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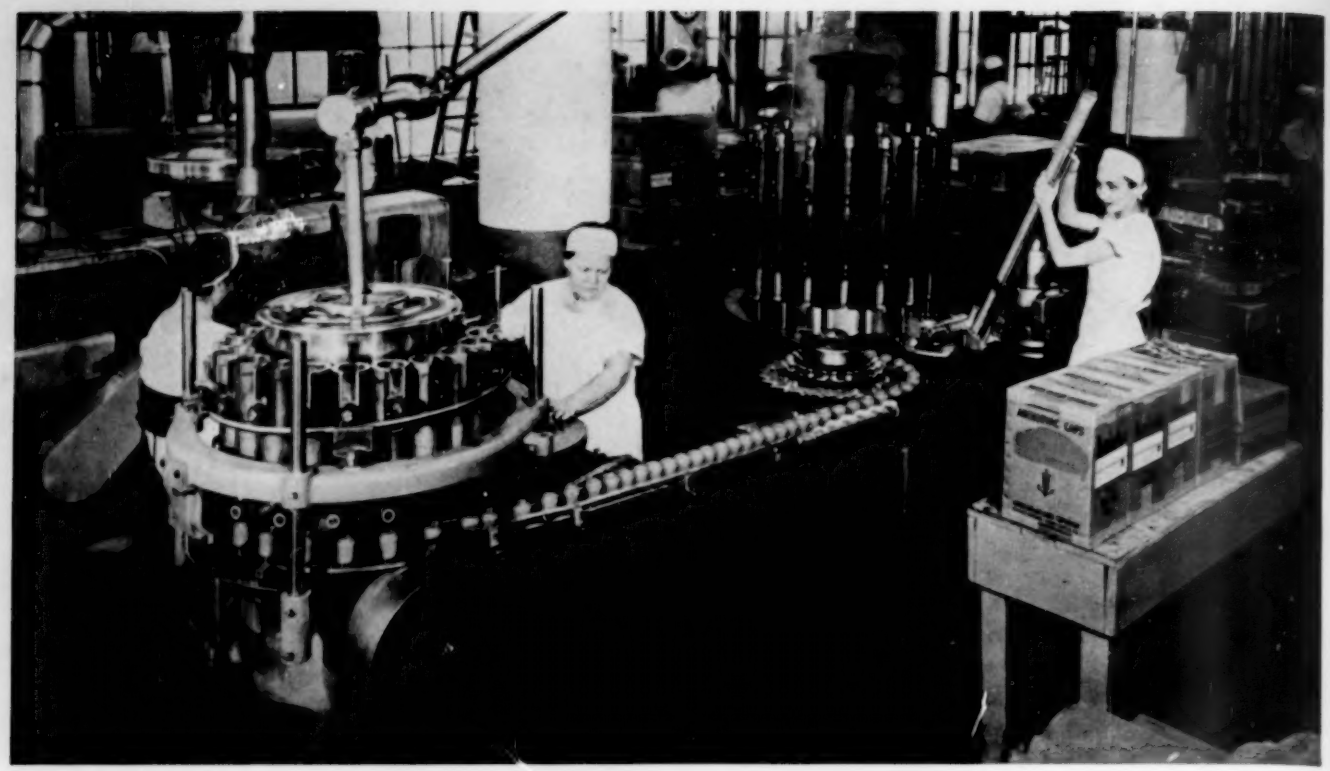

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Food Technology Curricula

This is the first of a series of articles which are intended to elucidate the basic ideas that govern the curricula of our educational institutions in the field of food technology. The author or authors of each article will belong to the staff of a Department of Food Technology which is actively training students. It is planned to include an article from each educational institution offering a curriculum in food technology. The article will describe the philosophy which guides the course of study and will describe the contents of the course. Included in the series later will be a report by the IFT Committee on Education, based upon the results of a questionnaire which was recently submitted to members of the IFT.

ILLINOIS INSTITUTE OF TECHNOLOGY

A Course of Study for Engineers for the Food Industry

J. H. RUSHTON [a] AND M. E. PARKER [b]

The food industries make up the largest group of the processing industries. Their dollar volume of product is the greatest. They cover the whole wide range of animal, vegetable, and fruit processing.

With the development of the factory system, food processing gradually has emerged from kitchen art to the technology of processing. The public health movement motivated the production development of thermal processing as it became apparent that disease bearing microorganisms could create widespread havoc unless brought under control. The science of bacteriology thus contributed to the advancement of food processing and distribution.

The acute emphasis applied to the furtherance of food preservation by thermal processing gradually revealed that the fundamental nutritional character of many processed foods had been thereby impaired. The discovery of vitamins and other vital accessory factors opened up entirely new vistas for the biological sciences.

Paralleling these developments, involving as they did the principles of bacteriology, nutrition and biochemistry, was the advent of chemical engineering as a profession, with its concept of unit operations.

Accordingly, men trained in chemical engineering have been entering food industries in increasing numbers because mass production techniques profited from the *know how* of the chemical engineer. And it soon became apparent that the food engineer should have not only the background of the chemical engineer but, in addition, must be familiar with the biological sciences which have a bearing on foods, their use, and distribution. It would hardly be sufficient to insert biology, bacteriology, and biochemistry courses in place of some of the less important ones of the chemical engineering curriculum and call the result a food option. Therefore, at Illinois Tech there has been initiated a curriculum called Food Engineering because we believe this will best serve the food industries and at the same time provide a high content of chemical engineering of top quality. This curriculum is administered within the Chemical Engineering Department, since the chemical engineering viewpoint presently provides the fundamental and essential guidance.

The curriculum in Food Engineering is outlined briefly in Table 1. It covers a normal four year college period, two semesters each year. It is evident from the table that the first two years are typical for a chemical engineering course of study except for the Zoology and Botany in the first year. Mathematics covers differential and integral calculus, and the chemistry courses are the major ones taken by chemical engineers and chemists. Five chemical engineering courses of the regular sequence are given and three courses (Food Technology, Food Engineering, and Food Analysis and Instrumentation) are specifically in the food area. A course in Bacteriology is given in the Junior year, and a course in the Economics of Food Production is given in the Senior year.

Brief descriptions of some of the major courses of the curriculum will help to show the subject matter to be covered and the philosophy behind its presentation.

Zoology and *Botany* provide training in the fundamentals of the economic and practical aspects of animal and plant life.

Bacteriology involves the study of microbes including bacteria, yeasts, and molds with due regard to their relation to sanitation, disease and immunity.

Biochemistry covers the carbohydrates, proteins, fats, enzymes, vitamins and other vital accessory factors of foods as well as processes such as digestion, absorption, etc.

Food Technology is concerned with classes and grades of foods, the biological, chemical and physical factors of food spoilage, and the principles of food preparation for processing as well as the effects of processing upon nutrition.

Food Engineering considers the unit operations and the processes involved in preparing food for commercial distribution, with particular emphasis upon preservation

[a] Professor of Chemical Engineering.
[b] Professor of Food Engineering.

TABLE 1

Undergraduate Curriculum in Food Engineering

Freshman		Sophomore		Junior		Senior	
1	2	3	4	5	6	7	8
Technical	Drawing						
English	English						
Zoology*	Botany*						
Math.	Math.	Math.	Math.				
General Chemistry	General Chemistry	Quantitative Organic Chem.	Organic Chem.	Physical Chem.	Physical Chem.	Biochemistry*	
		Physics	Physics				
			Mechanics				
			Ch.E. Stoichiometry	Ch.E. Unit Operations	Ch.E. Unit Operations Ch.E. Lab.	Ch.E. Lab.	
				Economics	Economics	Econ. of Food Production*	
				Bacteriology*		Food Technology*	Food Engineering* Food Analysis
				Technical Electives			
					Liberal Studies Electives		
							Government

*Courses not found in the regular chemical engineering curriculum.

techniques as well as the essential treatments of water supplies and other supplements utilized in food industries.

Food Analysis and Instrumentation is designed to acquaint the student with principal official methods for qualitative and quantitative analysis of food components.

Economics of Food Production acquaints the students with the economic and governmental forces at work that affect food production and the food processing industries.

Mechanics is a basic course in all types of engineering work. It takes up both statics and kinetics and gives necessary beckground to analyze problems of bodies or fluids at rest or in motion. It is also the background for the mechanical design of any structure or piece of process equipment.

The course *Chemical Engineering Stoichiometry* is the stoichiometry of fuels and combustion. It is the first of a chemical engineering sequence and emphasizes the material and heat balance. Extensive practice is given in setting up problems involving chemical changes and using the mole concept to effect quantitative solutions. Fuels and their combusion serve as source material but the course also shows how these principles are extended to chemical processes such as sulphuric acid manufacture.

The two courses in *Unit Operations* cover the background theory for such operations as fluid flow, heat transfer, distillation, extraction, mixing, filtration, and drying. Emphasis is placed on rate, and this, together with the material and heat balance, determines the rational basis for equipment size and design from the process point of view. Background along such lines is of increasing importance to the food industries. There are numerous opportunities to apply the theory of heat transfer, distillation, mixing, and the like to food processes, and many pilot plant operations are now in progress to apply these theories to improve such processes as oil-hardening, starch cooking, solvent extraction of oils, concentration of fruit juices, enzymic digestion and bacteria growth.

The *Chemical Engineering Laboratory* courses are designed to give experience in the handling of process equipment and measuring instruments, in planning experimental work, in the taking of experimental data, and in the appraisal of such data. All students handle heat exchangers, driers, pumps, stills and rectifying columns, mixers, filters, evaporators, and auxiliary equipment.

There are eight courses in the table marked with an asterisk. These are the courses mentioned above which are not contained in the regular Chemical Engineering curriculum. To compare this curriculum further with the one in Chemical Engineering at Illinois Tech, see Table 2. This shows the ten courses in the Chemical Engineering curriculum which are omitted in the food engineering curriculum. The two Chemical Technology courses are similar in all respects to the Food Technology and Food Engineering courses. They differ only in that the latter are directed almost entirely to principles and processes involving foods and pharmaceuticals. Of the remaining six courses of the Chemical Engineering curriculum, four of them may be taken, if desired, as technical electives. Table 2 shows also the recommended technical electives for the Food Engineering curriculum. These are shown in two groups. The first group is of an engineering nature; the second group is for the student who desires a knowledge of management.

This curriculum is now in its second year of operation and has students enrolled in each of the four years. The course of study is probably not yet stabilized and anticipated changes will probably be made. We have been aided in the establishment of the curriculum and courses

TABLE 2

Courses Not Included in the Chemical Engineering Curriculum

Freshman		Sophomore		Junior		Senior	
1	2	3	4	5	6	7	8
Zoology	Botany			Bacteriology		Biochemistry Econ. of Food Production Food Technology	Food Analysis Food Engineering
Chemical Engineering Curriculum Courses Not Included in the Food Engineering Curriculum							
				Chemical Technology		Chemical Technology	
						Ch. E. Thermodynamics	M. E. Thermodynamics
					Metallurgy		Process and Plant Design
				Electrical Engineering	Electrical Engineering	Equipment Design	Research
Recommended Technical Electives for the Food Engineering Curriculum							
				Electrical Engineering	Metallurgy	Ch. E. Thermodynamics	Food Process and Plant Design
				Accounting	Personnel Management	Theory of Management	Engineering Economics

of study to a considerable extent by an advisory council made up of prominent men in the food industries.[c]

Some students and some industrial firms are interested in the cooperative plan of education, whereby a student alternates periods of study with periods of employment in industry. Programs can be arranged, within the course of study of Table 1, for such cooperative work and approximately half the students now enrolled are on this basis. About ten food companies in the Chicago area have set up cooperative plans.

In addition to the undergraduate courses of study, work on the graduate level is about to be initiated. Several researches pertaining to food processing are now being carried on by graduate students in Food Engineering, and it is anticipated that such activities will grow as staff personnel with interest in food processing are added.

A special interest is being developed in the mass spectrometer as a research tool in the establishing of newer methods of analysis of volatile odors and flavors (both desirable and undesirable), detection of deterioration at earlier stages than by other objective methods, and detection and analysis of fermentation products. A service feature is extended to food processing companies permitting applied studies in the fields of food concentrates, food packaging, food acceptability, and the development and production of synthetic flavoring material. Such activities carried on with industrial and governmental cooperation will help Illinois Institute of Technology realize its educational and research object of enhancing and extending the quality control of food processing through its Food Engineering curriculum.

[c] F. C. Buzzelle, General Mills, Inc.; Berton S. Clark, American Can Company; George A. Crapple, Wilson and Co.; Victor Conquest, Armour and Co.; H. C. Diehl, Director, The Refrigeration Research Foundation, Inc.; A. L. Elder, Corn Products Refining Co.; George F. Garnatz, Kroger Food Foundation; Philip P. Gott, National Confectioners' Association; Charles E. Gross, John Morrell and Co.; Lloyd A. Hall, The Griffith Laboratories, Inc.; Colonel Rohland A. Isker, Associates Food and Container Institute; George L. Kanberg, United Air Lines; William G. Karnes, Beatrice Foods Company; John T. Knowles, Libby, McNeill and Libby; Norman H. Kraft, Kraft Foods Co.; Henry R. Kraybill, American Meat Institute Foundation; F. H. Kullman, Bowman Dairy Company; Colonel Paul P. Logan, National Restaurant Association; C. E. Maier, Continental Can Co.; Paul D. V. Manning, International Minerals and Chemical Corporation; Harold M. Mayer, Oscar Mayer and Company, Inc.; Roy D. Newton, Swift and Company; F. N. Peters, Quaker Oats Company; T. M. Rector*, General Foods Corporation; H. N. Riley, H. J. Heinz Company; J. G. Shakman, Pabst Brewing Company; Fred P. Siebel, Jr., J. E. Siebel Sons' Company; E. S. Stateler, Food Industries; Robert I. Tenney, Wahl-Henius Institute.

*Deceased.

Packaging Whipping Cream in Pressurized Containers[a]

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The development of single trip metal containers for pressurizing whipped cream is described. The history of such procedure, as well as the principle of whipping by effervescence, is outlined. Composition, packing procedures, and quality factors involved are discussed. Public health and safety considerations, as well as pressurized packing of other products, are considered.

[a] Presented in part at the Ninth Annual I. F. T. Convention, San Francisco, California, July 13, 1949.

This paper is concerned with the packaging in single trip metal containers of whipping cream with suitable

flavoring, stabilizer and propellant gases. Upon release of an attached valve and nozzle, the cream is dispensed in whipped form ready for table use. At present, the packaged cream is pasteurized only, requiring refrigeration storage.

As with most "new" products, it is the fruition or adaptation of several related but diversified processes or products. The production of whipping cream by means of "effervescence" instead of the mechanical incorporation of air, can be traced back through several decades and in recent years, has become quite familiar to the soda fountain trade. The gaseous dispersion of products from small containers became familiar to many as the aerosol insecticide "bomb" of World War II. A new post-war industry was launched by the use of can making equipment for the production of inexpensive single trip low pressure containers for such civilian items as insecticides, air deodorants, fire extinguisher fluids, etc.

The adaptation of such containers to the packing of pressurized whipping cream was a natural step. It is obviously wrong, however, to speak of the resulting product as "the adaptation of the aerosol principle" to a foam such as whipping cream. The term "pressure propelled products" appears best to describe this rapidly growing field.

What Is Whipped Cream?

Sommer *(25)* has described whipped cream as a dispersion of air or gas cells and clumped fat masses as contiguous phases in cream serum to form a more or less rigid foam. The more finely dispersed the gas and fat, the better the texture and rigidity of the foam. The difficulty in the use of whipping cream by the housewife is that in an effort to obtain adequate dispersion by mechanical whipping of air and clumped fat often the clumping is carried too far and churning failure results. The dairy industry has attempted to broaden the whipping range or plateau between adequate dispersion and excessive churning. An ample literature *(20, 16, 17, 14)* exists on the industry's effort to improve the quality of whipping cream by controlling the fat content, solids-not-fat, separating and pasteurizing temperatures, homogenization, aging, acidity, viscosity, sugar, stabilizers, as well as the temperature and type of whipping equipment.

All of the above factors are of some consequence in the production of whipping cream by "effervescence," but since by this method the most important factor of gaseous dispersion is better controlled, the pretreatment of the cream is of lesser significance. The erratic behavior of the housewife is replaced by an automatic effervescing.

Whipping by Effervescence

Effervescence is defined in Chambers Technical Dictionary as, "The vigorous escape of small gas bubbles from a liquid."

In whipping cream by effervescence, the gas is dissolved in the cream under pressure, and as indicated by Henry's Law, the solubility of a given gas in the cream is directly proportional to the pressures encountered. Upon release of the pressurized cream, through a suitable valve, to atmospheric pressure, the gas passes out of solution but tends to remain dispersed in the creamy mass as a foam. The amount of gas which escapes from solution depends upon the solubility of the gases used and the saturation pressure. Nitrous oxide alone or with carbon dioxide has been found to be within the desirable solubility range and at the same time both tasteless and innocuous.

A rapid review (mostly in the patent field) of the use of such gases for effervescing cream is of interest. The use of nitrous oxide and carbon dioxide for aerating drinks dates back at least to 1869 *(28, 15)*, and charging milk products to 1882 *(5, 13, 3, 26)*. More recently, Ashley *(2)* in 1925 described a process for charging and dispensing to a fluff, an ice cream mix under gaseous pressure. In 1932 Roush *(22)* prepared whipped cream by dispensing air under pressure in finely divided streams while the cream was gently stirred. Burmeister *(4)* in 1932 used both air and oxygen under pressure for foaming cream without mechanical whipping. During the period 1934 to 1936, Getz and Smith *(9, 10, 24, 11)* of the University of Illinois, produced whipping cream with 200 to 650% overrun by aerating with nitrous oxide and other gases in small containers at pressures of 75 to 90 p.s.i.g. In 1938, Reinecke *(21)* obtained a patent for a method of charging cream in a hermetically sealed container from a small nitrous oxide cartridge attachable to the sealed cream container. Such metal containers which can be charged with nitrous oxide from a small cartridge, similar to the classic charged water bottle, are now available commercially in the United States. In 1939 Diller *(6)*, and Goosmann *(12)* in 1941, described the use of nitrous oxide and carbon dioxide in producing whipped cream. Marmorek *(18)* at the same time described a charging device for the same purpose. Getz *(7, 8)* and Smith *(23)* in 1942 and 1948 obtained patents for the process of aerating butter fat containing liquids with nitrous oxide and other gases as well as apparatus for holding such pressurized products.

These developments have found commercial expression in the packaging of pressurized whipping cream in refillable metal containers distributed for the past ten years, mostly for drug store fountain and restaurant usage; a home reusable container chargeable with a cartridge, and a single trip low cost metal container for mass retail distribution. Distribution of this latter product was started in 1948 on a national scale and the estimated 1949 consumption is about 30 million units. Because it offers the greatest possibility of mass distribution and development for not only whipped cream but other food products, it will receive the attention of the remainder of this paper. The single trip container, thus far used for pressurized cream, is an adaptation of a seamless drawn steel beer can coated on the interior with a sanitary coating plus a microcrystalline paraffin type wax. The exterior is lithographed in color. The dispensing valve of rubber, stainless steel and/or plastic, is attached to a tinplate cap which is clinched with a suitable gasket to the top of the can.

Composition and Packing Procedure

The properly pasteurized cream (usually 30% butter fat or higher) is mixed in regular dairy equipment with sugar (5 to 10%), stabilizer and vanilla flavoring. The stabilizers are those common to the industry *(19)* e.g., dehydrated egg albumin, dehydrated egg yolk, sodium caseinate, gelatin, skim milk powder.

The mix (usually 7 fluid ounces) is then filled into the 12 fluid ounce pressure container on regular dairy fillers, the metal gasketed cap to which the valve has been assembled is then clinched to the top, after which the filled and closed cans pass to the gasser *(27)*. Whipping cream gas (either 100% nitrous oxide or with 15% carbon dioxide) is added through the valve directly from (through reducing valves) larger pressure cylinders of liquefied gas. The gassed cream is then shaken violently for 10 to 30 seconds. This shaking serves the dual purpose of hastening equilibrium of the gas between the cream and headspace and partially clumping the butter fat. Equilibrium pressures of 75 to 90 p.s.i.g. are usually obtained producing an average overrun of about 250% or yield of 25 fluid ounces of whipped cream from the original 7 fluid ounces. The industry is now in the process of converting over its unit operation to continuous automatic production.

Quality Factors

Overrun: The yield of whipped cream is of primary importance in determining quality and consumer acceptance. Overrun is calculated as follows:

$$\frac{\text{Volume Whipped Cream—Volume Liquid Cream}}{\text{Volume Liquid Cream}} \times 100 = \%\ \text{O. R.}$$

It is readily determined by weighing a given volume of whipped cream and comparing with the volume of an equal weight of liquid cream. Getz et al. *(11)* have shown that overrun is directly proportional to gas pressure. Our observation of commercial packs, as well as laboratory packs, substantiates this relationship though there is some can to can, as well as definite batch to batch, variation. However, the overruns obtained in our laboratory and observed in commercial runs were somewhat less than those obtained by Getz with equivalent pressures.

Getz et al. *(11)* data were apparently taken on the first fraction from the container. As might be expected, we have found a smaller overrun on later fractions from the same can. An average of 25 cans of 30% cream (mostly commercial, but some laboratory packs) dispensed with about 90 p.s.i.g. equilibrium gas pressure with 85% nitrous oxide and 15% carbon dioxide gave the following data:

First Fraction	268.9%
Second Fraction	251.0
Third Fraction	239.2
Fourth Fraction	218.9
Fifth Fraction	178.0
Sixth Fraction	138.5
Combined Total Overrun	211.9%

Since the internal pressure tends to drop as the contents are dispensed, this fractional variation in overrun appears unavoidable and does not appear to have lowered consumer acceptance of the product. A processor should set up necessary controls to obtain overrun in accordance with the demands of his market.

A word of caution relative to packing procedure is desirable. Some processors, in an effort to speed up their packing procedure, may shorten the gassing and shaking cycle and gas at higher headspace pressures of 100 to 125 p.s.i.g. expecting the remainder of the gas to be absorbed in time to reach the equilibrium pressure of about 90 p.s.i.g.

Experience has shown that the absorption rate of the gas without agitation is very slow. This was shown in a series of tests in our laboratory in which the cream was gassed without agitation at a headspace pressure of 160 p. s. i. g. and refrigerated with only ordinary movement at 40° F. (4.4° C.). Four weeks were required for an equilibrium pressure of about 80 p.s.i.g. to be reached. The whip first dispensed, therefore, was "wet" and unstable, but after vigorous agitation with no drop in gas pressure, a dry, firm whip was obtained. The gas used, as with all our experiments, was 85% nitrous oxide and 15% carbon dioxide.

The desirable packing procedure is to gas and agitate to obtain the final equilibrium pressure which gives the quality of product with overrun desired. The equilibrium pressure is that which does not diminish with further agitation. An ordinary pressure gauge with an adaptor for the particular valve used, permits a pressure reading with an insignificant (1 to 2 lbs.) loss of internal gas pressure.

By so checking his product at time of packing, the processor can vary his pressures on different quality creams from 10 to 20 lbs. to obtain an equilibrium product of uniform quality.

Rigidity and Drainage: A "dry," firm whip in which the "edges" of the cream formed by the flute on the dispensing nozzle remain sharp and distinct is desirable. Such a cream is smooth and uniform in texture with full body to the taste. A "wet" cream is associated with a low equilibrium pressure and insufficient agitation. Sommer *(25)* has associated this firmness with a tight network of gas bubbles interlaced with clumped fat particles. The initial agitation at time of packing, therefore, appears necessary to increase the rate of gas absorption, and also to produce this fine clumping.

Getz *(11)* has found the drainage to vary inversely as the butter fat content and the gas pressure. Drainage, however, is less of a quality factor than with the mechanically whipped cream, since the cream is normally consumed soon after dispensing. Drainage can be diminished by the addition of stabilizers which, however, also increase the viscosity of the liquid cream.

Homogenization and Viscosity: Homogenized cream classically has not been considered desirable for mechanically whipped cream. Getz et al. *(11)* reports that, "Homogenized cream whips equally as well as ordinary cream by the aeration process."

As can be expected, however, more agitation is necessary for homogenized cream, and in commercial practice on the single trip container where production rates are important, the practice has been away from the use of homogenized cream. However, in the final product, homogenized cream shows less tendency to creaming and plugging with less shaking required by the housewife before use.

TABLE 1

Bacterial Counts Packaged Cream, Gassed and Ungassed

Stored at 40-45° F. (4.4-7.2° C.)

Days	1-A (Gassed) [b]		1-B (Ungassed) [b]		2 [c]		3 [d]	
	Bacteria/cc.	Flavor	Bacteria/cc.	Flavor	Bacteria/cc.	Flavor	Bacteria/cc.	Flavor
0	150-5,900 Ave. (11 Cans) 2,700	....	130	....	150-5,900 Ave. (11 Cans) 2,700	....		
7	107,000-506,000 Ave. (7 Cans) 237,000	....	40,000,000	....	10,000,000 (a) 13,700 (b)	....	107,000-506,000 Ave. (7 Cans) 237,000	O.K.
14	4,700-520,000 Ave. (7 Cans) 189,300	O.K.	169,000,000	Off	41,700,000 (a) 13,100,000 (b)	O.K.	1,060,000 (a) 570,000 (b)	O.K.
15	360,000	O.K.	159,000,000	Off	60,000,000 (a) 3,700,000 (b)	Off	68,000,000 20,000,000	Off
16	1,560,000	O.K.	120,000,000	Off	14,800,000 670,000	Off	4,800,000 (a) 319,000 (b)	Off
21	3,100,000	O.K.						
28	8,900,000	O.K.						
35	12,400,000	Off						

[b] Series 1-A and 1-B—Counts on individual cans first dispensed at time of count.

[c] Series 2—Gassed—All cans partially dispensed on 0 day—Individual cans of this group dispensed and counted on successive days. (a) Count is on first gram dispensed; (b) on second fraction.

[d] Series 3—Gassed—All cans partially dispensed on 7th day—Individual cans of this group dispensed and counted on successive days. (a) Count first gram; (b) second fraction.

The excessive use of stabilizers in commercial practice, which tends to decrease drainage, may so increase viscosity as to also slow down absorption of the gas. Likewise, excessive viscosity tends to increase internal foaming or gelling in dispensing and some residual cream may be retained in the container.

A more thorough study of the effect of homogenization as well as the specific effect of stabilizers is desirable as an aid to processors. Processors of pressurized cream, however, should seek to better understand the principles of whipping by effervescence and work out satisfactory control practices with a given formulation and varying cream quality.

Public Health and Safety Considerations

The metal containers for pressurized cream are manufactured and shipped so that they comply with the regulations of the U. S. Public Health Service Sanitation Advisory Board.

These regulations *(27)* state that, "all single service containers and container caps and covers shall prior to use be so processed as to produce containers having a residual bacterial plate count of not more than one per cc. of capacity, and caps or covers with not more than 10 colonies each, as determined by the rinse technic described in the latest Standard Methods."

A bacteriological survey of a typical processor showed the following data, the mix having previously been pasteurized for 30 minutes at 180° F. (82° C.):

Cream Mix from Bulk Can—80-90 Bacteria per cc.
Cream Mix from Filler Bowl—420 Bacteria per cc.
Cream Mix from Uncapped Cans—190-5400 Bacteria per cc.
Rinse Count of Empty Cans—2-32 Bacteria per can
Rinse Count of Caps and Valves (after chlorine treatment)—8-10 Bacteria per cap

At the time the above survey was made, data was collected on the effect of gas (85% N_2O and 15% CO_2) on the preservation of the product.

The results are given in Table 1. Though there was considerable can to can variation in the count, the data in Series 1A (gassed) and 1B (ungassed) clearly indicate the bacteriostatic effect of the gas upon the cream. These series were on cans stored and counted without previous dispensing. Series 2 and 3 indicate the effect of dispensing and then storing. Once the product is first dispensed, the count and deterioration increase more rapidly thereafter. Likewise, a higher count is indicated in the stem of the valve (a) than in the later portion dispensed (b).

Relative to the non-toxicity of nitrous oxide, Getz et al. *(11)* have reviewed the literature and conclude that it is widely used as an anesthetic because of its non-toxic effect on the animal system. It is only an anesthetic in high concentration of 90-95% and obviously the small amount consumed is insufficient to act as an anesthetic. Only 5 or 6 grams of nitrous oxide are used in producing a quart of whipped cream.

For any pressurized product, safety during shipment and in the hands of the consumer must be carefully considered. To fully evaluate this factor, the pressure-temperature relation of a given formulation must be known. If, at the maximum temperature to which the product will normally be subjected, the pressure is well below the failure range of the container, it can be assumed that it can be marketed with safety.

Temperature-pressure curves for typical pressure whipping cream are given in Figure 1. The upper curve with an equilibrium pressure of 97 p.s.i.g. at 40° F. (4.4° C.) gives a pressure of 150 p.s.i.g. at 130° F. (54° C.). The temperature of 130° F. (54° C.) is the upper reference temperature normally used by the I.C.C. in evaluating pressure containers. Since processors usually do not exceed 95 p.s.i.g. at 40° F. (4.4° C.) and the pressurized cream is held under refrigeration, the possibility of the product being subject to these upper temperatures and pressures is remote. However, containers should provide safety even under extreme conditions. The single trip container which is now used for pressurized cream will withstand about 175 p.s.i.g.

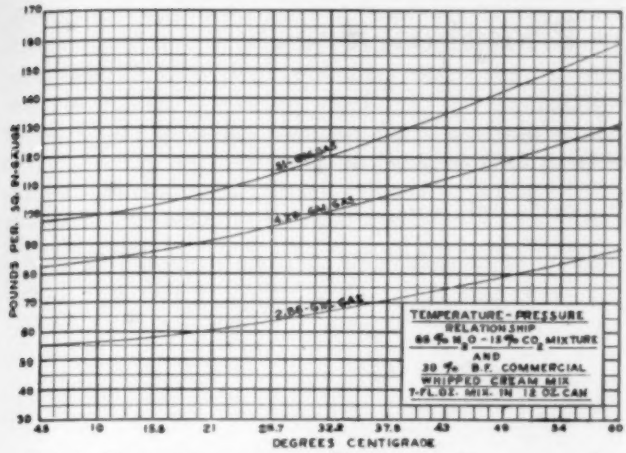
POUNDS PER SQ. IN. GAUGE
DEGREES CENTIGRADE
TEMPERATURE - PRESSURE RELATIONSHIP
88 % N_2O - 12 % CO_2 MIXTURE
AND
30 % B.F. COMMERCIAL WHIPPED CREAM MIX
7 FL. OZ. MIX IN 12 OZ. CAN

FIG. 1. Temperature-pressure relationship of pressure packed whipped cream mix.

or higher before deformation. At this pressure, the concave bottom usually everts, but leakage does not occur until about 225 p.s.i.g. or higher. These containers are drawn, i.e., "seamless" without the soldered side seam which is the weakest point of ordinary sanitary food or beer cans.

After due investigation and receipt of pressure-temperature data, the I.C.C. has issued special permits for the shipment of pressurized cream in single trip containers. These special permits are in lieu of regular tariff regulations which are forthcoming.

Application to Other Products

The use of pressurized containers for other food products is of interest and already has created considerable enthusiasm. A few fundamental considerations are necessary, however, to keep this enthusiasm within limits. In general, three types of pressurized products can be produced:

1. *Mists or Fine Sprays:* Although this form of dispersion is readily applied to many insecticides, pharmaceutical and cosmetic products, it seems reasonable that there is little application to food products except possibly in the flavoring field.

2. *Foams:* Foams such as whipped cream appear to be the best form of application in the food industry. Possible additional products are salad dressing, mayonnaise, marshmallow, meringue, pancake batter, and others.

3. *Non-Aerated Products:* Such products as catsup, mustard and chocolate syrup immediately suggest themselves. It would appear questionable, however, on these products whether the advantages would be worth the added packaging problems.

Once a product is selected for pressurized packaging, considerably more is involved than merely placing in a can, gassing and turning the valve. To the contrary, each "pressure propelled" product offers a different problem which can only be solved by the proper consideration of the following factors:

1. State or condition of dispensed product; that is, whether a fine mist or spray, foam, or non-aerated product is desired.

2. Formulation—Usually each product has to be reformulated.

3. Valve design and construction, which will produce the desired dispensation.

4. The compressed gas or propellant with proper pressures and solubilities.

5. The container with proper lining and satisfactory service life.

In summary, we report that the packaging of pressurized whipped cream in single trip metal containers appears certain to become a standard dairy item offering a wholesome cream product in convenient form to the American family. Likewise, the use of this method for other food products bears investigation by food technologists.

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A Micromethod for Detecting the Enzymatic Breakdown of Cephalins; and/or Phosphoserines[a]

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A diffusion procedure is described in which enzymatic digests are oxidized with periodate in a Petri dish, and the ammonia so formed is diffused into sulfuric acid spread on the ground surface of the glass plate cover of the Petri dish. The diffused ammonia is estimated by nesslerization. The adequacy of the method was demonstrated with standard ethanolamine solutions and also with an alkaline hydrolyzate of synthetic palmitolinoleocephalin.

As the method measures only those free bases that form ammonia with periodate, it is specific for the liberation of ethanolamine or serine, and so distinguishes phosphatidyl ethanolamines and phosphatidyl serines from the lecithins.

The method clearly demonstrated the existence of an enzyme system in cabbage juice, capable of liberating ethanolamine or serine from a phospholipide, but unable to split synthetic cephalin.

The occurrence of phospholipides in food products is very widespread, although in only a few instances (such as soybeans or egg yolk) do they constitute an appreciable proportion of the total lipides. Admittedly the presence of even small amounts of phospholipide in foods and equally their removal by possible enzymatic breakdown, can affect the consistency of the material, its emulsifying properties, its susceptibility to oxidation and rancidity, and thus in all probability, its taste.

The fate of phospholipides in most agricultural products has received little attention. The reasons for this are first that the identity of the particular phospholipides in a given product is not always known, and second that methods for demonstrating their enzymatic breakdown leave much to be desired. Recently, however, Hanahan and Chaikoff *(4)* have demonstrated the presence of phospholipide-splitting agents in carrots and cabbage by showing a decrease in the amount of ether-soluble nitrogen, after the enzyme had acted. Since aqueous extracts of these vegetables also digested soybean "lecithin" with the liberation of choline, it may be concluded that lecithin was among the phospholipides decomposed. But since the free choline found did not account for all of the nitrogen made insoluble in ether during the digestion, it seems likely that some other phospholipide was also attacked in the soybean preparation (which is known to be a mixture). By the methods in current use it would be very difficult to determine analytically which of the several constituents of soybean "lecithin" had been hydrolyzed; and pure samples of all the individual phospholipides are so difficult to obtain that their use as test substrates is not practical in most cases.

The scheme proposed here is based upon the fact that serine and ethanolamine alone of the *known* basic components of phospholipides liberate ammonia on oxidation with periodate. The method is therefore highly specific. It measures only the formation of ethanolamine and serine, thus indicating only that particular decomposition of cephalins or phosphoserines, respectively, that leads (directly or indirectly) to the liberation of the base itself.[c]

Periodate oxidation has been used by several workers for the quantitative estimation of free ethanolamine *(1, 2, 3, 5)* and of serine *(1, 3, 8)*. However, one difficulty encountered with most agricultural products is that they foam badly during the distillation of ammonia. This difficulty has been overcome by the use of very small quantities of material in a modification of the well known microdiffusion apparatus, after which ammonia was estimated with Nessler's reagent. Under the conditions described free ethanolamine reacted quantitatively to form ammonia. Ethanolamine existing as part of the cephalin molecule did not react at all, as shown by the behavior of synthetic cephalins *(10)* of known composition. The behavior of serine with periodate has been described already by Nicolet and Shinn *(8)*.

Diffusion Apparatus. The diffusion apparatus is similar to that employed by Prater, Cowles and Straka *(9)*. It consists of a 100 x 15 mm. Petri dish with a ground edge, and a cover plate with a ground surface. As cover we employed the bottom plate of a micro bell jar, of a type used for vacuum filtration of small quantities of materials. Before use the outer annulus of the ground surface is rubbed with a small amount of silicone stopcock grease and the excess is wiped off. This prevents the drop of sulphuric acid on the central portion of the cover from creeping to the edge during the diffusion and the washing. One application of silicone lasts through many cleaning operations. Silicone, however, is not necessary to prevent a loss of ammonia.

Analytical Procedure

A glass swab is prepared by wrapping a small amount of glass wool around one end of a glass rod. This is dipped in 20 percent sulfuric acid and wiped over the central portion (5-6 cm. diameter) of the ground surface. The enzyme digest, and 2 ml. of saturated potas-

[a] Presented in part before the Ninth Annual Institute of Food Technology Convention, San Francisco, California, July, 1949.

[b] Enzyme Research Division Contribution No. 123.

[c] There are several ways in which a phospholipide may hydrolyze. One (possibly both) fatty acids might be removed, leaving the base still attached to phosphoric acid and this in turn to glycerol. Or the phosphorylated base might be separated from the glycerol skeleton. (The ester perhaps to be further hydrolyzed to free acid and base.) A third case would be the liberation of the base only from the rest of the molecule. Enzymes have been reported to catalyze each of the foregoing changes, whether separately is not known in all cases. It seems entirely possible that all these changes might occur in the same product, as they seem to ultimately when a phospholipide is digested by an animal.

sium metaborate are placed on one side of the Petri dish, and 0.5 ml. 2 *M* periodic acid is placed on the other side. The cover plate is then put on and the contents are mixed by rotating carefully. The dish is then left for 3 hours at room temperature, after which the cover plate is removed and the liquid on the ground surface is washed into a 10 ml. volumetric flask. A 2 ml. aliquot of this solution is transferred to a 25 ml. flask, 7.6 ml. water is added, and then 0.4 ml. of Nessler's reagent [Jackson's modification *(11)*] is added to this solution. The flask should be well agitated by swirling [d] during the addition of the reagent. After 5-10 minutes the percent of transmission of light is measured on the nesslerized solution with an Evelyn colorimeter using filter 520. A reagent blank is prepared by wiping the sulfuric acid swab on the inside of a flask, adding 9.6 ml. water and 0.4 ml. Nessler's reagent. The instrument is adjusted to read 100 with this solution.

The amount of ammonia nitrogen found may be read from a curve prepared with standard ammonium chloride solutions containing 5 to 50 micrograms of nitrogen. Under the conditions described here the chromogen obeys Beer's law over this range. If the amount of ammonia is greater than 50 micrograms, the colorimetric determination is repeated with a smaller aliquot of the diffusate and a correspondingly larger quantity of water. If below the range indicated, it is repeated, with a more concentrated solution of the diffusate. Good results were obtained with an 8.0 ml. aliquot of 10 ml. diffusate, and, addition of 1.6 ml. water and 0.4 ml. of Nessler's reagent.

The results obtained with aqueous solutions of freshly distilled ethanolamine are given in Table 1. The first column lists the amount of ethanolamine used for reaction with periodate (and for diffusion) expressed as nitrogen.

Enzymatic Cleavage of Phospholipides. Attempts were made to demonstrate the presence of an enzyme capable of liberating ethanolamine or serine from the corresponding phospholipides. The results are cited here because they justify the method and particularly its specificity; as a study of the zymolysis of phospholipides they are a preliminary report.

As substrates the following were employed:

(A) Synthetic dipalmito- or palmitolinoleocephalin was dissolved in acetic acid (50 mg. per ml.), neutralized with 8 *N* NaOH and 2 percent sodium glycocholate solution added (10 ml. per 50 mg. cephalin).

[d] During the course of this work, two papers *(6, 7)* appeared in which the factors influencing the determination of ammonia by nesslerization were reported in considerable detail. These papers indicate that it is necessary to have good agitation during the addition of Nessler's reagent, and that the amount of (concentrated) sulfuric acid in the solution to be nesslerized must not exceed 0.3 ml. The amount of sulfuric acid used to trap the ammonia on the cover plate was well below the above limit. However, if Nessler's reagent was added without vigorous agitation, turbid solutions were sometimes obtained if the amount of ammonia present was large. Miller and Miller *(7)* employ mechanical agitation during the addition of Nessler's reagent. This has the advantage of uniform treatment, but we obtained good results by swirling the flask vigorously by hand.

TABLE 1

Recovery of Ethanolamine from Pure Ethanolamine and from Mixtures with Dipalmitocephalin (Expressed as Micrograms of Nitrogen)

Ethanolamine nitrogen for diffusion	Ethanolamine nitrogen for nesslerization	Nitrogen found	Recovery percent
57.3	57.3	60	105
229.4	114.7	115	100
229.4	229.4	223	97
114.7 [e]	114.7	109	95
114.7 [e]	114.7	115	100
114.7 [e]	91.8	96	104
229.4 [e]	229.4	227	99
59.0 [f]	59.0	61	103
59.0 [f]	59.0	58	98
59.0 [f]	59.0	61	103

[e] Added to bile salt solution containing 50 mg. of undecomposed dipalmitocephalin in absence of enzyme preparations.

[f] Palmitolinoleocephalin hydrolyzed by heating on the steam bath for 15 minutes with 4 *N* sodium hydroxide to effect hydrolysis. Aliquots representing 15 mg. of the original cephalin were assayed.

(B) Two commercial samples of soybean phosphatides,[g] suspended in 0.05 M phosphate buffer, pH 5.9 (100 mg. per 2.5 ml.). One of these (A in Table 2) consisted of crude soybean phospholipides, the other (B in Table 2) had been purified by extraction with acetone, which removed glycerides.

Enzyme preparations were made from carrot and cabbage as described by Hanahan and Chaikoff *(4)*.

TABLE 2

Digestion of Phosphatides by Enzymes of Cabbage Juice

	Nitrogen liberated micrograms			
	4 hours		12 hours	
	Total	From action of enzyme on phosphatide	Total	From action of enzyme on phosphatide
Cabbage enzyme alone	1	....	6	....
Phosphatide A	10	....	6	....
Phosphatide B	0	....	8	....
Palmitolinoleocephalin [i] (PLC)	81	....	92	....
Enzyme + A	237	227	625	613
Enzyme + B	340	329	575	561
Enzyme + PLC	106	24	162	64
Enzyme + A [h]	5	....	....	....
Enzyme + B [h]	0	....	....	....
Enzyme + PLC [h]	0	....	....	....

[h] No periodate added for diffusion.

[i] In bile salt solution. Impurities in the bile salts are doubtless responsible for the appreciable blank.

The carrot preparation had no appreciable effect on any of the substrates but the cabbage preparation readily modified the soybean lipides in such a way that ammonia was evolved with alkaline periodate, but not with alkali alone. Unfortunately so much formaldehyde was liberated from the sugar present in the cabbage juice that turbidity occurred with the Nessler's reagent. It therefore became necessary to purify the cabbage enzyme in order to eliminate the sugar. This was accomplished by saturating the cabbage juice with sodium chloride and filtering off the precipitated proteins, among which is the enzyme. The proteins were redissolved in water so as to be 10 times as concentrated as in the original juice. Table 2 gives the results of two experiments (typical of several) showing the effect of the cabbage preparations when reacted with

[g] We wish to express our appreciation to Mr. John H. Lathe, Manager of Lecithin Sales, The Glidden Company, Chicago, Ill., for supplying the two samples of soybean phosphatides.

the various substrates for 4 and 12 hours respectively at room temperature (25°). In these experiments 20 mg. of synthetic cephalin or 100 mg. of crude soybean phosphatides were treated with protein equivalent to 10 ml. of cabbage juice. The mixture was placed directly in the diffusion dish described previously, and the alkaline periodate solution added after the stated incubation interval.

It will be noted that the behavior of the cabbage enzyme is quite curious. There was no effect upon the synthetic cephalins. On the other hand, the soybean lipides (which undoubtedly also contain cephalin) reacted readily. There is thus presumptive evidence that cabbage contains an enzyme that liberates serine from phosphoserine, rather than ethanolamine from cephalin.

Summary

A method for the microdetermination of ethanolamine and serine is described which is suitable for detecting the enzymatic hydrolysis of cephalins or phosphoserines when the free base is liberated. The method has been proved on synthetic cephalins of known composition.

With this method it was observed that a crude preparation of protein from cabbage juice decomposed a mixture of soybean phosphatides liberating either ethanolamine and/or serine. The preparation did not affect synthetic cephalins in a similar manner. There is thus presumptive evidence of the existence of an enzyme in cabbage capable of splitting serine from phosphatidyl serine.

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Processing and Packaging of Dates. I. A New Method of Canning and Pasteurizing Deglet Noor Dates[a]

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A method of canning dates, involving loose packing, addition of a small amount of water, replacement of atmosphere with nitrogen, and pasteurization has yielded products of excellent flavor and color. Data obtained on color changes and spoilage are reported.

Introduction

Most dates retailed commercially at present are marketed in packages wrapped with transparent films. Dates maketed in this manner seldom average more than 25 percent of moisture by weight, and they have a tendency to become increasingly dark during their shelf life as a result of action of oxygen in the air. It has been considered possible that a new and desirable date product could be created with economic advantage to the industry if a method were developed for preserving dates of about 30 percent moisture content so that they would remain free from spoilage and retain a desirable color and texture.

An obvious approach to this end would be pasteurization of dates in hermetically sealed containers. Fellers *(3)* has pasteurized dates in cellophane-wrapped cartons and Postlethwaite *(5)* has reported the preservation of dates in hermetically sealed containers by pasteurization under vacuum. Both methods of preservation appear to be highly satisfactory for the purposes intended; however, the method of Fellers does not provide for the exclusion of oxygen, and certain undesirable changes in texture and appearance were found to accompany the application of Postlethwaite's method to dates of 30 percent moisture content.

The experiments reported in this paper were designed: (1) to develop a process that would give a product with good color, appearance, and flavor; (2)

[a] Presented before the Ninth Annual I. F. T. Convention, San Francisco, Calif., July 13, 1949.

[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

to investigate one of the causes of spoilage, and (3) to develop a pasteurization process that would prevent spoilage.

Retention of Natural Color and Appearance in Pasteurized Dates

Color changes ("browning") in pasteurized dates have been commonly attributed to the caramelization of sugars and to the Maillard type of browning reaction involving reducing sugars and amino acids. Accordingly, experiments were initiated to study the inversion of sucrose in Deglet Noor dates caused by pasteurization and to determine the relationship between invert sugar and brown color development in these dates. Unless otherwise stated, all experiments were performed with Deglet Noor dates having an average of 30 percent moisture content and which had been sprayed with an infusion of fermenting date tissue.

Invert sugar was determined by the Lane-Eynon procedure *(1)* and color was estimated by a procedure somewhat similar to that used by Stadtman et al. *(8)*, for determining brown colors in dried fruit products. A 25-gram sample of mixed date tissue was macerated for 10 minutes with 200 ml. of a 50:50 ethanol-water mixture in a Waring Blendor,[c] filtered, and the absorption of the filtrate was read in a Klett photoelectric colorimeter at 420 mμ. Color readings thus obtained are only relative and do not show the color differences as markedly as they appear on visual inspection.

In one typical experiment green Deglet Noor dates were packed in air and under vacuum in 211 x 400 enamel-lined cans, pasteurized in boiling water for 15 and 30 minutes, and stored at 85° F. (29.4° C.). The samples were opened in 30 days and examined for color and invert sugar content.

In Table 1 little relationship between amount of sugar inversion and color of dates is apparent. Although both of the atmospherically-packed samples show more inversion and greater color than corresponding vacuum-packed samples, it is apparent that the atmospherically-packed sample No. 4, with an invert sugar content slightly less than that of vacuum-packed sample No. 3, is close to twice as dark in color.

It was concluded that darkening of pasteurized dates is primarily an oxidative reaction, perhaps involving the date tannins, rather than a caramelization or a Maillard-type reaction. The results also indicated that dates can be pasteurized without seriously affecting color if oxygen is excluded by some method such as use of vacuum.

[c] The mention of special instruments throughout this paper does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

TABLE 1

Effect of Pasteurization on Color and Invert Sugar Content of Dates Stored at 85° F. (29.4° C.)

Sample No.	Storage Time, Days	Type of Pack, 211 x 400 Cans	Pasteurization in Boiling Water, minutes	Invert Sugar, Fresh Weight %	Colorimeter Reading at 420 mμ
1	None	Control (not canned)	Fresh dates	12.0	120
2	30	Vacuum	15	15.5	132
3	30	Vacuum	30	17.8	140
4	30	Atmospheric	15	17.3	242
5	30	Atmospheric	30	21.8	254

Although dates pasteurized in a vacuumized container were found to retain a desirable appearance with regard to color, it was noted that the use of vacuum had a deleterious effect on the textural appearance of the dates. Such dates often appeared bloated, and the skins were badly split and loosened. The flesh appeared gassy, as though fermentation had occurred, although odor and flavor were normal. This effect is peculiar to the higher-moisture dates and is practically absent in dates of 25 percent or less moisture content similarly pasteurized under a vacuum.

To retain the advantages of eliminating oxygen from the container and to avoid the rupturing effect of pasteurizing dates in a vacuum, it was decided to do further work on packing dates under a vacuum released with nitrogen before pasteurization. A large pack of choice hydrated Deglet Noor dates was made in which samples were packed (1) under vacuum, (2) under vacuum released by nitrogen (99.5 percent pure), and (3) atmospherically. All samples were pasteurized 30 minutes in boiling water and stored at 90° F. (32.2° C.). Samples were examined at various times for color, texture, and invert sugar content (Table 2).

The data again demonstrate the effect of oxygen on the color changes that dates undergo during storage. The pasteurized, atmospheric packs showed the greatest color development. A comparison of invert sugar content and color after 105 days' storage shows rather clearly that invert sugars are not involved to any great extent in color formation. It is also apparent that release of vacuum with nitrogen is effective in preventing a ruptured appearance in these dates.

TABLE 2

Color, Texture, and Invert Sugar Changes in Stored, Processed Dates

Type of Pack 211 x 400 cans	Texture	Storage Time in Days, at 90° F. (32.2° C.)					
		1		59		105	
		Colorimeter Readings at 420 mμ	Invert Sugar, Fresh Weight, %	Colorimeter Readings at 420 mμ	Invert Sugar, Fresh Weight, %	Colorimeter Readings at 420 mμ	Invert Sugar, Fresh Weight, %
Control	....	114	19	....	....	....	....
Vacuum	Ruptured	116	25	110	27	125	29
Vacuum	Ruptured	114	23	114	25	127	26
Nitrogen	Normal	116	24	113	26	118	28
Nitrogen	Normal	117	25	113	26	120	27
Atmospheric	Normal	101	24	127	26	135	28
Atmospheric	Normal	115	24	131	26	136	27

It is concluded from this work that the color and appearance of dates can be successfully preserved during and after pasteurization by processing in a vacuumized container, with the vacuum released by nitrogen (or other inert gas) prior to pasteurization.

The observed darkening in these experiments does not appear to be the result of caramelization or the Maillard reaction, as has been commonly supposed. The effect of vacuum and nitrogen in preventing darkening suggests a primarily oxidative mechanism. Dates are known to contain relatively large amounts of tannin *(10)*, which is mostly located in a layer of giant cells just under the skin of the dates. During pasteurization, changes take place in this layer that ultimately lead to "browning" if the dates are stored in an oxygen-containing atmosphere. Upon pasteurization, even under nitrogen, this layer acquires a rusty red color which does not show through the skin of the dates sufficiently to alter markedly the overall color. However, when such dates are stored in an atmosphere containing oxygen, the color of this layer becomes greenish-black and shows through the skin, rendering their appearance undesirable.

It is possible that the red pigment consists of phlobaphenes formed from the date tannins as a result of heat applied during pasteurization. The red pigment is considerably more sensitive to oxidation than the naturally occurring tannins in the fresh date. This can be easily demonstrated by treating the giant cell layer of heated and fresh dates with a drop of dilute ferric chloride solution, or by moistening with dilute ammonium hydroxide and exposing to air. In either case the red-colored giant cell layer of the heated date turns greenish-black instantly, whereas the corresponding brown-flecked layer of the fresh date becomes darker more slowly. This difference is apparently not a matter of availability of tannins to the reagents, since macerated date tissue, heated and unheated, shows the same behavior.

A Factor Governing Spoilage in Dates

In our experimental work we soon found that, while date packers had difficulty preventing spoilage, we had difficulty in obtaining it consistently. Since it was necessary that our unpasteurized control samples spoil, a study was made to determine the conditions necessary for spoilage. We had observed that dates having a high percentage of moisture invariably spoiled and with that in mind, tests were conducted to determine at what percentage of moisture spoilage would occur and at what percentage it would occur *rapidly*.

The choice, natural Deglet Noor dates used in this experiment had been commercially graded and packed in a 15-lb. flat box. Individual dates were selected and checked for moisture content on a refractometer *(6)*. The skin was partly peeled back, the moist tissue smeared on the refractometer, and the reading taken and converted to percent moisture. The moisture contents ranged from 21.9 to 31.5 percent, but only dates testing 24 to 31.5 percent were used. To the open surface of the fruit, a small portion of fermenting date pulp was then added, the skin was folded back into place, and the date was sealed in a small cellophane bag. The samples were stored at 80° F. (26.5° C.) with 80 percent relative humidity until spoilage occurred.

Dates with low moisture contents (Figure 1) fermented only after 23 days' storage, whereas dates with a high moisture content started fermenting after 6 days. This is in agreement with the belief of date packers that low moisture content dates are more resistant to spoilage.

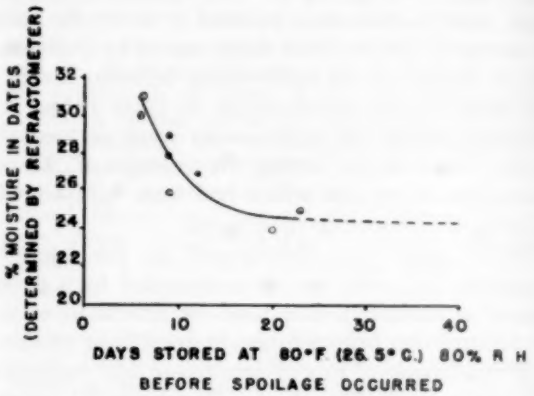

FIG. 1. Effect of Moisture on Rate of Spoilage in Choice, Natural, Deglet Noor Dates. (The moisture content was determined on the refractometer to the nearest full percentage. The following number of dates from each percent moisture were tested: 31% — 3; 30% — 4; 29% — 7; 28% — 10; 27% — 7; 26% — 7; 25% — 12; 24% — 3.

In considering the curve in Figure 1, it may be seen that as moisture decreases, the days before spoilage occurs increase. The curve theoretically approaches a line asymptotically somewhere below 25 percent moisture, where no spoilage occurs.

Although the figure clearly demonstrates the effect of relative moisture content on keeping quality, the occurrence of spoilage in lower-moisture dates in less than one month is somewhat misleading. The experimental dates were stored at 80 percent relative humidity and, according to Rygg *(7)*, dates tend to equilibrate their moisture content with the relative humidity of the surrounding atmosphere. It is probable that the dates having 24 to 25 percent moisture absorbed moisture during storage at 80 percent relative humidity until the moisture content in the date became sufficiently high for spoilage to occur. We have, in fact, stored dates of 25 percent moisture content at lower relative humidities for more than six months without encountering spoilage.

An interesting point is that these dates had been commercially graded as having a moisture content of about 24 percent, although refractometric determinations showed that moisture content actually ranged from 21.9 to 31.5 percent. Since high moisture dates fermented within a few days, it is possible that a few high-moisture dates in a box or carton may initiate the spoilage that causes serious losses to the packer. Apparently a more reliable method of grading dates according to moisture content would benefit the packers and it might be possible to eliminate spoilage completely in cartoned dates by excluding any high-moisture (over 25 percent) dates and avoiding storage at high temperatures and humidities.

Developing the Pasteurization Procedure for High-Moisture Dates

Dates are a relatively solid fruit of high sugar and low-moisture content, and since they are usually packed without a sirup, it is to be expected that the rate of heat penetration would be low and that longer pasteurizing times would be required than for products packed in brines, juices, and sirups. Therefore, heat penetration studies were conducted with the dates packed in 211 x 400 enamel-lined cans under conditions of vacuum, nitrogen, and atmosphere. These dates contained an average of 30 percent moisture and were sprayed with an infusion of fermenting date tissue.

Small, flexible thermocouples and seals *(4)* were employed which could be inserted into the air spaces in the cans or directly into the dates in order to make the temperature measurements. Pasteurization temperatures of 170°, 212°, and 240° F. (76.5°, 100°, and 115.5° C.) were initally tested, but a study of heat penetration at 240° F. (115.5° C.) was not carried out since dates processed at this temperature were too sirupy and developed a dark coloration which was not prevented by the replacement of air with nitrogen. The heat-penetration curve in Figure 2 shows that the temperature of 170° F. (76.5° C.) was too low for our purposes, since the internal temperature of the date did not reach 140° F. (60° C.) after even 45 minutes' pasteurization. [The temperature of 140° F. (60° C.) was taken as the *minimum* that would destroy yeast cells *(9)*.] A pasteurization temperature of 212° F. (100° C.) was therefore chosen for all subsequent studies, since, as will be shown, at this temperature the appearance of the dates was not adversely affected, heat penetration was considered reasonably rapid, and this temperature was convenient to achieve and maintain.

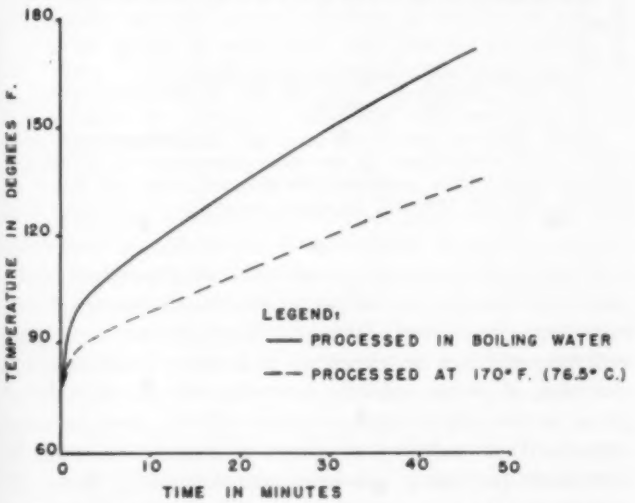

FIG. 2. Heat Penetration of Dates in 211 x 400 cans (temperatures taken inside center date).

Other tests were made to determine the effect of adding a small amount of water (1 ml.) to the can before sealing, since water vapor might transfer heat more rapidly through the can during pasteurization. The effect of the resulting water vapor on heat penetration in dates (Figure 3) was only slight; however, the effect on the temperature of the air space was marked. Not

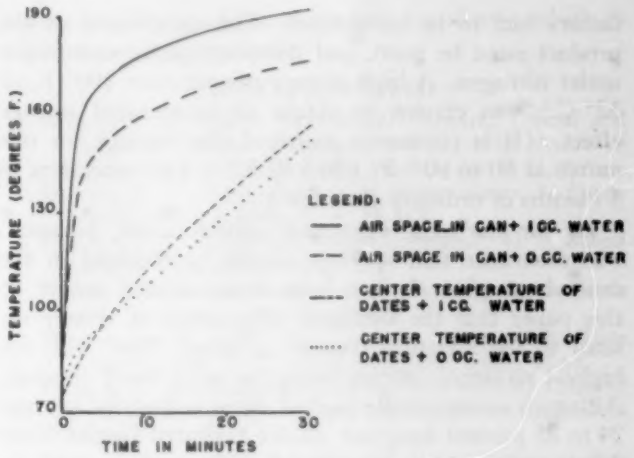

FIG. 3. Effect of Added Water on Processing Temperature in 211 x 400 Cans of Deglet Noor Dates (all cans processed in boiling water).

only did the temperatureof the air space increase more rapidly with the addition of moisture, but the final temperature reached in the air space in 30 minutes was 15 degrees higher than that in the air space in cans having no added water. Since most of the organisms capable of causing spoilage might be expected to be found at the surface of the date, the addition of moisture to the pack would seem to be a worthwhile precautionary measure, particularly since the further advantage of hydration can be obtained. The appearance of the dates is not affected by this treatment.

Water cooling of the cans after processing of course lowered their temperature much more quickly than air cooling (Figure 4). If it is desired to use water cooling in the process, it would seem advisable to increase the minimum process time to obtain the same pasteurizing effect. Water cooling apparently had no beneficial effect on the appearance of the pasteurized dates, and it therefore seems that air cooling should be a preferred procedure because of the added "kill" of other organisms no doubt obtained while the product is still at a relatively high temperature. Air cooling should of course be under conditions allowing free circulation and the cooling time should not exceed two hours before the product has reached room temperature.

In order to determine the pasteurization procedure best suited to the preservation of dates, a number of

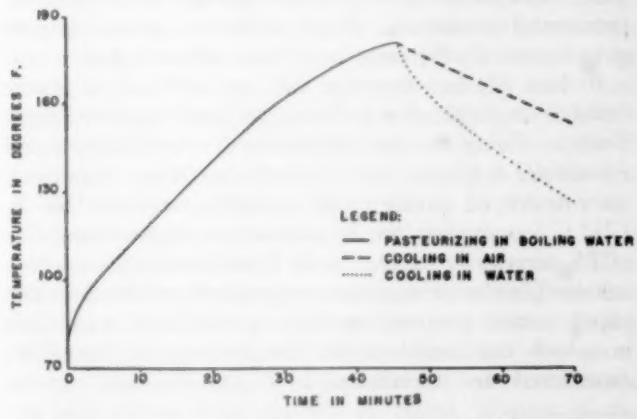

FIG. 4. Water vs. Air Cooling with 211 x 400 Canned Deglet Noor Dates (temperatures taken inside center date).

factors had to be considered. The appearance of the product must be good, and therefore packs were made under nitrogen. A high storage temperature (90° F. or 32° C.) was chosen to obtain an accelerated storage effect. (It is commonly assumed that storage for one month at 80 to 90° F. (26.5 to 32° C.) is equivalent to 3 months of ordinary shelf life.)

By far the most important consideration, however, was assurance that spoilage would be obtained in the control samples. It has been demonstrated earlier in this paper that the incidence of spoilage is closely related to the moisture content of dates, those with the highest moisture content being the most likely to spoil. Although commercially packed dates ordinarily average 24 to 25 percent moisture, choice hydrated Deglet Noor dates containing approximately 30 percent moisture were chosen for these experiments. This moisture content was attained by soaking the experimental dates in water 15 to 30 minutes and then placing them back into 15-lb. flat boxes, and storing at 38° F. (3.5° C.) for 2 days to equalize the moisture content. In addition, they were sprayed prior to the experiments with the infusion of spoiled fermenting date pulp. Under the storage conditions employed, unpasteurized controls sprayed with this infusion invariably spoiled within two weeks' storage time.

In one experiment the dates were packed in 211 x 400 cans sealed under vacuum released by nitrogen and pasteurized 15 minutes, 30 minutes, 45 minutes, and 60 minutes in boiling water. Approximately 24 cans of each variable were processed and held for storage. The cans were considered fermented or spoiled when they began to swell. In addition, cans were "cut" each month and examined organoleptically for other types of spoilage.

The results after storage are shown in Table 3. Dates preserved by pasteurization in boiling water for 30 minutes or longer and stored at 90° F. (32° C.) have remained free from spoilage up to the time of publication of this paper.[4]

The results of the pasteurization experiments with respect to spoilage are supported by numerous other similar experiments not reported here in detail. In all cases of packing under vacuum, nitrogen, or atmosphere, and with or without the addition of water to the can, a 30-minute pasteurization treatment in boiling water was sufficient to prevent spoilage under the experimental conditions. Dates packed in carbon dioxide gave essentially the same results as with nitrogen.

Fellers *(2)* has reported that an outbreak of severe "colitis" in England was traced to dates imported from France. Using the test organisms *Escherichia coli* and *Eberthella typhosus,* he reported that these organisms were destroyed on dates held at a temperature of 160° F. (71° C.) or higher for 30 minutes at relative humidity of 75 percent or more. Since these conditions are not achieved in the pasteurization treatment reported in this paper, which prevents spoilage, a test pack was made in which the inside center temperature of the dates (measured by thermocouples) was brought up to 165° F. (74° C.) by pasteurization in boiling water for 45 minutes and the canned dates were then transferred to a 165° F. (74° C.) bath and held for an additional 30 minutes. Figure 5 shows that the conditions specified by Fellers are thus met and examination of the dates indicated that they corresponded in color and appearance to those packed under nitrogen in boiling water for 30 minutes. Thus, should dates prove a factor in the transmission of disease in this country, the holding treatment at a lower temperature may be employed for more complete sterilization without impairment of quality.

[4] Packed February, 1949.

TABLE 3

Effect of Pasteurization in Boiling Water on Microbial Counts

Pasteurization Time, Nitrogen Pack	Number of Cans	Microorganisms Immediately after Processing (per gram) *	Spoilage, %	Storage Period
15 minutes	24	287	100	2 weeks
30 minutes	24	100	0	9 months
45 minutes	24	No count	0	9 months
60 minutes	24	No count	0	9 months

* The average microbial count before processing was 80,000 organisms per gram. Bacterial counts were made as follows: A single date was aseptically weighed and placed into a sterile pint jar containing 90 cc. of sterile, distilled water. After soaking for one hour, the jar was shaken vigorously two minutes to disintegrate the date. Dilutions were made and 1 ml. of each of various dilutions was plated, with dextrose tryptone agar containing brom cresol purple indicator. The plates were incubated 48 hours at 90° F. (32° C.) and counted. Although these microbial counts may be subject to error because of the method of sampling, it is probable that reasonable results were obtained. The count before processing is the average of four samples. The counts obtained after processing were from only one sample of each variable tested, but the samples were taken from the center of the cans and their count would tend to show whether the processing was ineffective.

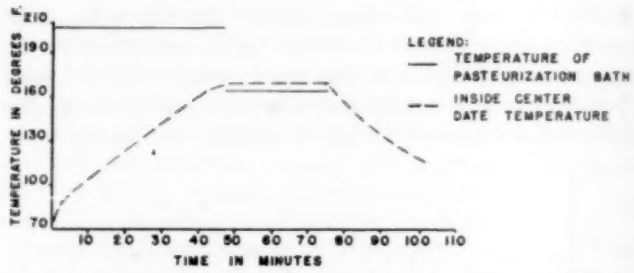

FIG. 5. Rate of Heat Penetration with Dual Pasteurization of Dates in 211 x 400 Cans. (Cans were pasteurized 45 minutes in boiling water, then processed 30 minutes more in water at 165° F., (74° C.) followed by water cooling).

Since dates are not a markedly acid product (i.e., pH 5.5-7.0), the possibility of Botulinus infection has also been considered. Although the pasteurization procedure could not be expected to destroy Botulinus organisms, it seems unlikely that they would be able to grow at the high sugar content (65-75 percent total sugars, fresh weight) of dates. Certainly no case of Botulinus poisoning has ever been traced to dates, although 50 lb. compressed blocks of dates, which have essentially anaerobic interiors, have been an item of commerce for many years. Similar non-acid products such as sirups are not considered sources of Botulinus poisoning after pasteurization, partially by virtue of their high sugar content, which is of the same magnitude as that of dates. However, it is suggested that anyone contemplating canning dates by this process should obtain clearance on this point from Federal and State Public Health authorities.

Summary and Conclusions

1. Packed dates of excellent flavor and color may be satisfactorily canned by (1) loosely packing about 7 ounces of dates into a 211 x 400 enamel-lined can, (2) adding approximately 1 ml. of water, (3) vacuumizing (28 inches), (4) releasing with nitrogen gas of 99.5 percent purity, (5) closing the can, (6) pasteurizing 30 to 45 minutes in boiling water, and (7) air cooling so that room temperature is attained in about two hours. Pasteurization for 45 minutes in boiling water is preferred for the added margin of safety thus afforded. Dates packed in cans other than 211 x 400 should, of course, be pasteurized for different lengths of time.

2. Darkening in dates appears to be caused by oxidative reactions involving tannins and products resulting from the action of heat on these tannins. These reactions are inhibited by packing dates in an inert atmosphere such as nitrogen or carbon dioxide.

3. The length of time before spoilage occurs in dates is inversely related to their moisture content, and as moisture percentage increases, the length of time before spoilage occurs decreases.

4. Pasteurizing high-moisture dates under a vacuum causes a splitting of the skins and a puffiness which is unattractive in appearance.

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The Ultraviolet Absorption of Polyphenolic Substances from Various Fruits[a]

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Ultraviolet absorption of polyphenolic substances from various fruits is presented. The two absorption bands (280 and 324 mμ) obtained are due to two different types of substances. The band at 324 mμ is due to caffetannin which resembles chlorogenic acid. The relationship between UV absorption and the amount of polyphenols estimated colorimetrically is also discussed.

Introduction

Ultraviolet absorption spectroscopy is frequently used as a tool in aiding the identification of certain organic compounds or chromophoric groups. This paper discusses the application of ultraviolet absorption technique to the study of the polyphenolic substances extracted from certain fruits. This method proved to be very useful in showing the presence of two types of phenolic substances (tannin materials) in peaches and other fruits. Peaches, apples, cherries, apricots and other fruits contain polyphenolic substances which are important from the standpoint of flavor (astringency) and enzymatic oxidation or browning. These polyphenolic substances are the natural substrates involved in enzymatic browning of certain fruits. The work which has been done in relation to the characterization or identification of these natural substrates is very meager. Onslow *(3)* referred to them as catechol tannins because of the tests they gave for the catechol grouping. Lavollay, Sevestre and Dussy *(1)* reported the presence of catechin in various fruits.

Materials and Methods

Fruit samples which were specially frozen for the extraction of the polyphenolic substances were used. Apple slices, unpeeled peach halves, Italian prune halves, and pitted cherries were packed in a No. 10 standard enamel tin can and frozen immediately after packing. The fruit was stored in the frozen state to be extracted at a more convenient time. At the time of sampling, the fruit was ground in the frozen state with an ordinary meat grinder and then placed back into freezer storage (—22° C.). This ground material was used for the extraction. The peaches were slip-peeled before grinding after immersing the halves for about 10-15 seconds in cold water. The fruit was handled in the above manner to prevent any thawing so that no significant enzymatic browning would take place.

For the extraction of the phenolic substances, 100 g. samples of the frozen ground fruit were weighed into a Waring Blendor cup. The sample was extracted with 400 ml. of 70 percent ethyl alcohol for about four minutes. Approximately 200 ml. of the extract were filtered through fluted filter paper. A 50-ml. aliquot of the filtrate was pipetted into a 100-ml. centrifuge tube.

[a] Scientific Journal Series No. 298, Colorado Agricultural Experiment Station, Colorado A. & M. College, Fort Collins, Colorado.

[b] A portion of this work was done in partial fulfillment of requirements for a Master of Science degree.

Three to 5 ml. (depending upon the tannin concentration) of 0.5 M neutral lead acetate were added to precipitate the polyphenolic substances. All of the phenolic materials were not precipitated under the above conditions. There was a variation of approximately 80 percent in the case of peaches to less than 60 percent in the case of Bing cherries. The mixture was centrifuged for 5 to 10 minutes at approximately 2000 r.p.m. The clear supernatant liquid was decanted off the lead precipitate. The precipitate was then washed with 20 ml. of 70 percent alcohol containing small amounts of lead acetate. After centrifuging and decanting, the washing operation was again repeated. Five ml. of 1 N H_2SO_4 were added to decompose the lead precipitate to form lead sulfate. The phenolic materials are dissolved in the dilute H_2SO_4 solution. This mixture was centrifuged and the liquid decanted through a small filter paper to insure a clear liquid. The lead sulfate was washed with 5 ml. of 1 N H_2SO_4 and the above procedure repeated. The filter paper was washed with 20 ml. of 1 N H_2SO_4. The filtrate containing the phenolic substances and other material precipitated by lead was made to 50 ml. volume with distilled water. This solution was diluted ten or more times for the ultraviolet absorption determinations which were made on a Beckman Model D U Quartz spectrophotometer, using 1 cm. quartz cuvettes and a hydrogen discharge lamp as the light source. All of the optical density values are expressed on the same basis, namely, the density of the above 50 ml. solution diluted 10 times. The amount of phenolic material in the above extract was determined as tannic acid using the method of Rosenblatt and Peluso *(4)*. The total amount of tannin in the alcoholic extract was also determined to obtain the amount which was precipitated with lead acetate under the above conditions.

Results and Discussion

Figures 1 and 2 show the UV absorption of the lead precipitated phenolic substances extracted from several peach varieties containing varying quantities of tannin. Two maxima were obtained, one at 280 mμ and the other at approximately 322 to 324 mμ. The fact that the ratio of these two peaks varies with different fruits and different varieties of the same fruit indicates that there are two types of substances present which possess definite chromophoric differences. A maximum in the region of 280 mμ is typical for phenolic compounds which do not have a chromophoric group conjugated with the benzenoid structure. A chromophoric group conjugated with the benzenoid structure may result in a bathochromic shift. Chlorogenic acid which has maximum adsorption at 324 mμ is an example of a compound in which this type of shift has occurred. Chlorogenic acid can be considered a substituted catechol. Catechol has an absorption maximum of 278 mμ.

In Figure 3 are shown the absorption curves of phenolic substances from various apple varieties. The curves are very similar to the peach curves. The UV absorption curves of Montmorency cherry, Italian prune and Bing cherry are distinctly different from the peach or apple curves. They are shown in Figure 4. In the case of Montmorency cherry the 324 mμ absorption is relatively low while it is exceptionally high in cases of

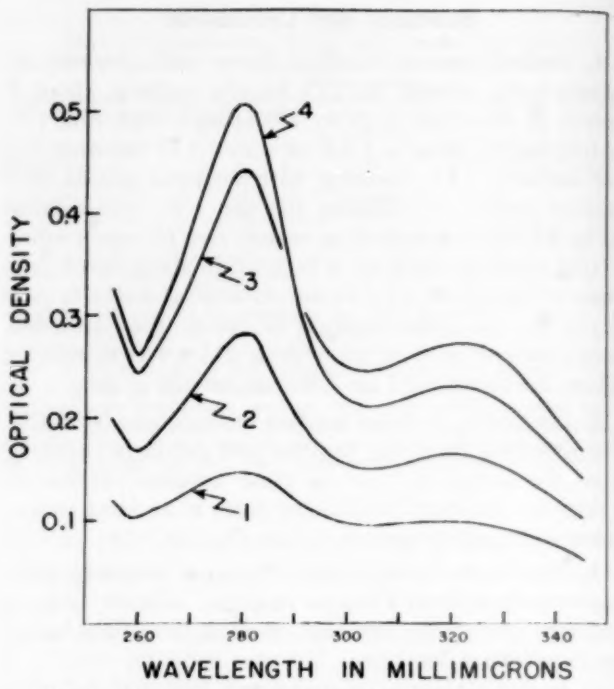

FIG. 1. Absorption spectra of peach phenolic substances.
1. Canadian Queen. 2. Rio Oso Gem. 3. Elberta and 4. J. H. Hale.

Bing cherry and the Italian prune. Apparently these fruits contain more of the substances contributing to the 324 peak than the other fruits.

An excellent relationship was found between the amount of phenolic substances precipitated by neutral lead acetate as determined colorimetrically according to the method of Rosenblatt and Peluso *(4)* and the optical density at 280 mμ of the same extract. This relationship is shown in Figure 5. With apples, peaches and Montmorency cherry, the coefficient of correlation was found to be +0.98. The above relationship does not apply in

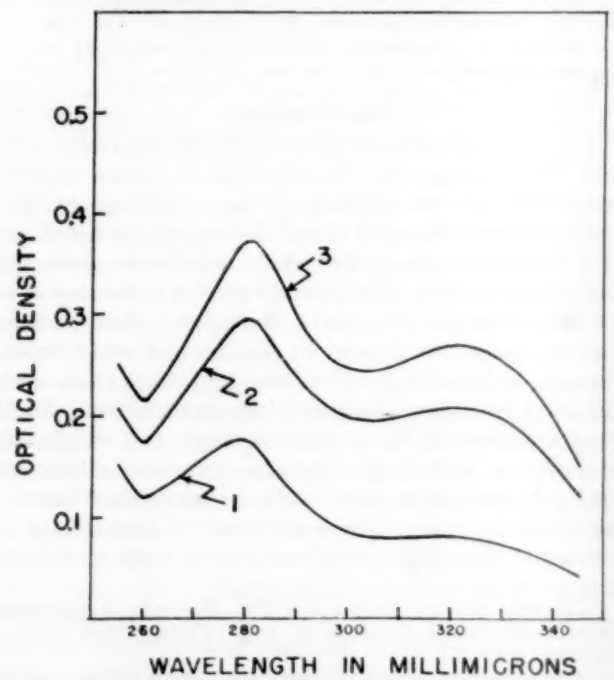

FIG. 2. Absorption spectra of peach phenolic substances.
1. A seedling. 2. Hale Haven and 3. Globe Haven.

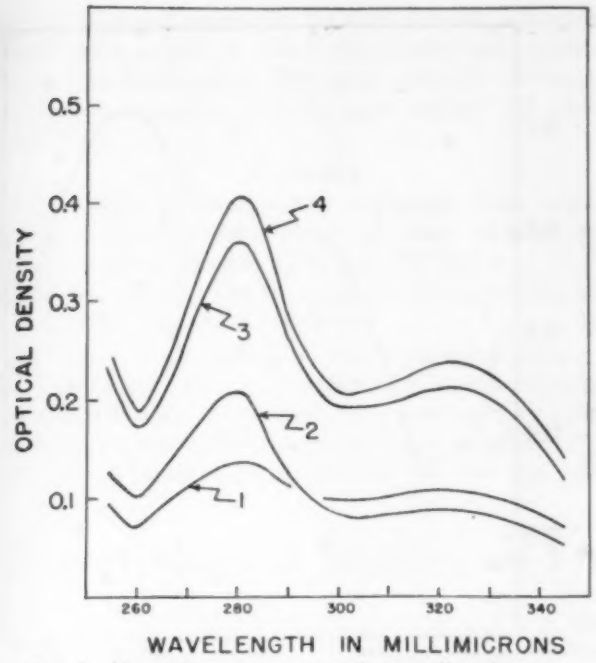

FIG. 3. Absorption spectra of apple phenolic substances.
1. Blackwine. 2. Jonathan. 3. McIntosh and 4. Turley.

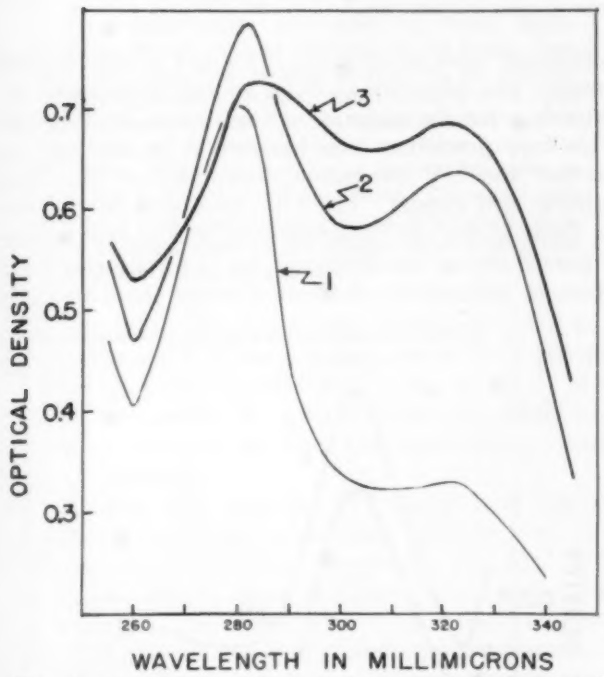

FIG. 4. Absorption spectra of cherry and prune phenolic substances.
1. Montmorency cherry. 2. Italian prune and 3. Bing cherry.

case of Bing cherry and Italian prune. The UV absorption was found to be too high compared with the other fruits. This may be due to the fact that large amounts of anthocyanin pigments are precipitated.

An experiment was conducted to note the effect of adding chlorogenic acid to Elberta peach phenolic extract (lead precipitated). The results are shown in Figure 6. Curve 1 is the absorption curve for chlorogenic acid, hemihydrate [c] concentration being 0.1

[c] Kindly supplied by R. G. Moores, Central Laboratories, General Foods Corporation.

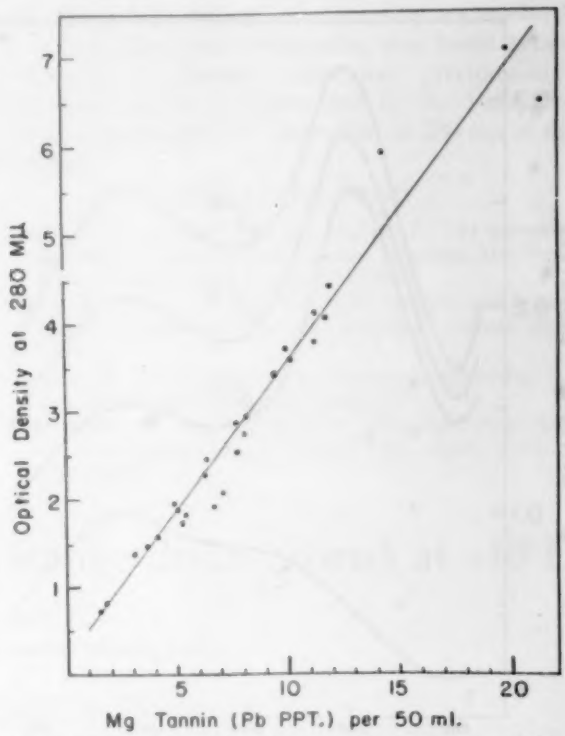

FIG. 5. The relationship between UV absorption at 280 mμ and the amount of tannin determined colorimetrically.

mg./50 ml. of very dilute sulfuric acid. Curve 2 represents the Elberta peach phenolic substances (0.79 mg./50 ml. as tannic acid). When the above amount of chlorogenic acid was added to the peach extract, absorption curve 4 was obtained. When one-half the above amount of chlorogenic acid was added, curve 3 was obtained. It will be noted that curves 3 and 4 are similar to those of Italian prune and Bing cherry.

A synthetic anion exchange method [d] was used to isolate crude polyphenolic substances from Elberta peaches. This material had UV absorption very similar to the lead precipitated peach phenolic extract. The phenolic material was subjected to paper chromatography using 77 percent ethyl alcohol as the developing solvent. It was resolved into three components. One component having a R_f value of about 0.65 fluoresced vivid blue when the paper chromatogram was exposed to UV light with maximum illumination at 3650 A°. Furthermore, this substance gave greenish yellow fluorescence upon exposure to ammonia fumes and UV light. Chlorogenic acid behaves in exactly the same manner and has the same R_f value as the above material. Water or alcoholic solution of chlorogenic acid does not fluoresce. However, when a drop of the solution is allowed to dry on a filter paper, the spot will fluoresce blue and greenish yellow when exposed to ammonia fumes.

Another substance which was not distinguishable on the chromatogram under UV light became visible when the chromatogram was treated with ammonia fumes. The substance fluoresced blue. D-catechin has very similar characteristics and both substances have the same R_f value of about 0.75. The third material which

[d] A future paper will report this method.

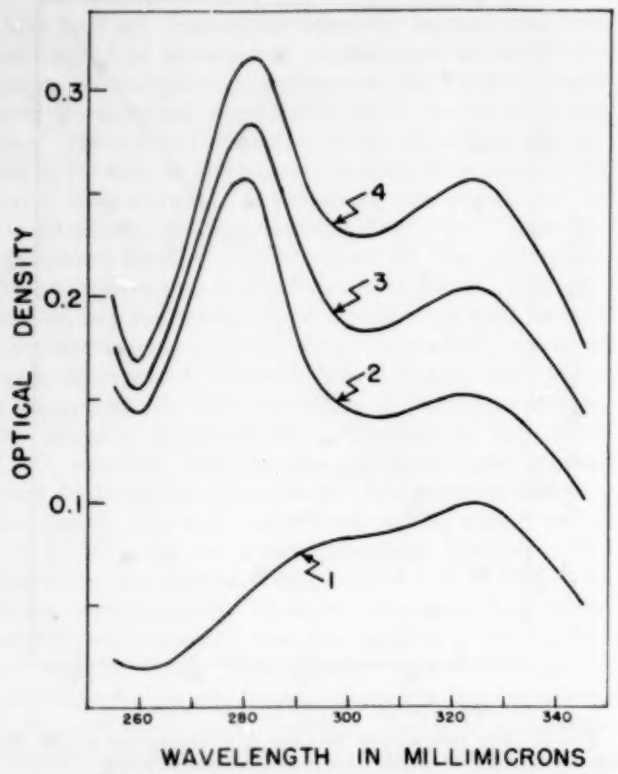

FIG. 6. The effect of adding chlorogenic acid to peach phenolic substances.

1. Chlorogenic acid. 2. Peach phenolic substances. 3. and 4. Peach phenolic substances with added chlorogenic acid.

was somewhat diffused on the paper chromatogram was reddish brown in color (day light). It possessed no fluorescence even after exposure to ammonia fumes. The R_f value was less than that of the other two substances. Sufficient quantities of the above materials were separated upon a paper chromatogram so that they could be extracted and UV determination made on the extracts. The substance with an R_f value of 0.65 and fluorescing blue and greenish yellow after exposure to ammonia fumes gave an absorption curve very similar to chlorogenic acid with a maximum at 324 mμ. Sometimes it was necessary to chromatograph this material twice to obtain good separation from substances having a maximum at 280 mμ. Figure 7 shows the UV absorption spectrum of a peach caffetannin extracted from a paper chromatogram. Both the other two substances gave a maximum at 280 mμ. It is postulated by the authors that the reddish brown substance may be phlobotannins. This substance gave a green color with ferric chloride.

The above work substantiated the fact that the two maxima—one at 280 mμ and the other at approximately 322-324 are due to two different types of substances. The latter peak is due to a caffetannin which resembles chlorogenic acid. This type of tannin has not previously been reported to be present in such fruits as peaches and apples. Mrak, Fessler and Smith *(2)* isolated caffeic acid from alkaline hydrolyzed water-extract of prunes.

As previously stated, neutral lead acetate does not completely precipitate the fruit phenolic substances under the conditions used. In an experiment using Elberta peaches, it was found that all of the phenolic

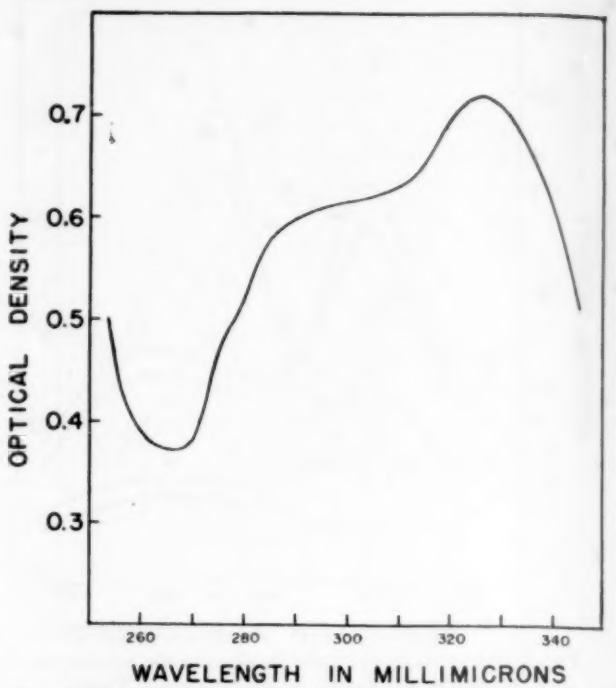

FIG. 7. Absorption spectrum of a peach caffetannin extracted from a paper chromatogram.

substances were precipitated if the pH of the alcoholic extract was adjusted to 8 by adding ammonium hydroxide after the addition of the neutral lead acetate. This lead precipitate was handled as in the previous manner and UV absorption determined on the dilute sulfuric acid extract. The UV curve was very similar in shape to the one obtained with neutral lead acetate. Figure 8 shows the absorption curve for Elberta peach phenolic substances precipitated under these two con-

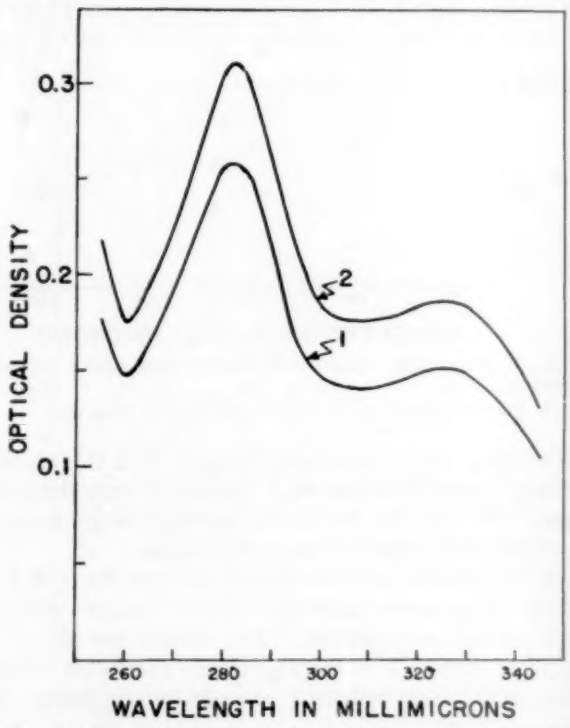

FIG. 8. Absorption spectra of peach phenolic substances.

1. Precipitated with neutral lead acetate. 2. Made alkaline with NH_4OH to pH 8.

ditions. Curve 2 represents the total phenolic substances precipitated at pH 8. This experiment was repeated using Jonathan apples. The apple phenolic substances were also completely precipitated under the above conditions.

Summary

1. Polyphenolic substances extracted from apples, Italian prunes, peaches, sweet and sour cherries give two absorption maxima (280 and 322-324 mμ) in the ultraviolet region. The two maxima are apparently due to two different ypes of substances.

2. The UV absorption of peach phenolic substances at 322-324 mμ is due to a caffetannin which resembles chlorogenic acid. Both have very similar UV curves and behave similarly on a paper chromatogram. Two or more substances contribute to the 280 mμ peak. D-catechin and its derivatives apparently are involved.

3. A high significant relationship was found between the amount of phenolic substances precipitated by neutral lead acetate as determined by the Folin-Denis phenolic reagent and UV absorption at 280 mμ of these substances.

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Bacteriological Studies on Frozen Orange Juice Stored at -10°F.[a]

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Total plate counts were made on frozen single-strength orange juice at intervals for 43 weeks. During this time total counts dropped in an approximately logarithmic manner. Coliform tests were made by standard methods. Various organisms that met the definition for coliform persisted in frozen juices for 43 weeks. Most were Aerobacter species, but some resembling Escherichia coli were found. The significance of these organisms remains to be determined, since tests were not made on the interior or exterior of the fruits before they were juiced.

Earlier bacteriological studies on orange juice have been concerned with the possible role of the organisms in bringing about deterioration of the product. More recently the question of what coliform organisms were present in frozen orange juice has stimulated a number of investigations.

Nolte and von Loesecke *(8)* found that coliform bacteria were present in numbers varying from 0 to 1,000 per cc. of raw juice. Teunisson and Hall *(14)*, Patrick *(9)*, and Wolford and Berry *(21)* studied bacteria isolated from machinery in the lines of citrus processing plants and found *Aerobacter aerogenes* to be a commonly occurring organism in such equipment. A few intermediate coliforms but no *Escherichia coli* were found.

Wolford and Berry *(20)* showed that the juice produced from soft-rot fruit contained many times the total bacteria numbers and coliform density found in juice of sound fruit. These differences persisted through 8 months' storage at 0° F. (—17.5° C.). The coliforms isolated were largely members of the *Aerobacter* genus.

The survival of *E. coli* inoculated into experimental packs of frozen orange juice has been reported by McFarlane *(7)*, who found that the initial plate count of 3 to 8 million per cc. changed to 3 per cc. in one week of 0° F. (—17.5° C.) storage. He reported that in solutions of orange juice and sucrose, the organism remained viable much longer at 0° F. and that total numbers of viable cells increased with increasing sucrose concentration in the range investigated.

In attempting to answer the question whether *E. coli* will survive subfreezing temperatures, Kieth *(6)* found that this organism died rapidly when frozen in tap water, but remained alive for many months when frozen in sherbet ice. When suspended in aqueous mixtures of glycerine and held at —20° F. (—28.5° C.) a large proportion of the cells remained alive for at least 6 months. Weiser and Osterud *(19)* found that freezing is more lethal than super-cooling and that the rate of death is much greater with freezing temperatures above —22° F. (—30° C.) than with temperatures below this point.

This paper reports bacteriological findings on juice frozen and stored at —10° F. (—23° C.) for periods of 2 to 43 weeks. This work was undertaken to provide information concerning naturally occurring microorganisms in single-strength frozen orange juice and the survival of these organisms during storage in frozen condition as measured by plate counts and standard coliform analyses.

Source of Packs Studied

Four packs of juice produced in three different plants were studied. Processing-grade California Valencia oranges were used in each pack. The data concerning the plant conditions at the time the packs were prepared are presented in Table 1. Each pack was taken from a single batch of juice. Twenty-four No. 2 tins of juice made up Pack A; 24 No. 1 tins, Pack B; 23 No. 1 tins, Pack C; 8 one-gallon tins, Pack D. The total number of the samples was thus 79.

[a] Presented before the Ninth Annual I. F. T. Convention, San Francisco, Calif., July 12, 1949.

[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE 1

Conditions Under Which Packs of Juice Were Prepared

Pack	Plant	Apparent Sanitation of Line	Type of Extraction Employed	Apparent Condition of Fruit
A	1	Good [c]	Hand [d]	Poor. Small fruit with a number of soft discolored oranges.
B	2	Fair [e]	Machine	Sound.
C	2	Fair [e]	Machine	Poor. Soft discolored fruit in lot.
D	3 [g]	Good [f]	Machine	Sound.

[c] Slimes not detected on equipment in line. Shallow bins used to reduce injury to fruit.

[d] Attempts were made to eliminate contamination from workers' hands by requiring help to wash hands and follow with a sanitizing dip in quaternary ammonium solution.

[e] Slimes detected on conveyor belts, wash tanks, and sizers. Deep, inadequately baffled bins did not protect against crushing the fruit.

[f] Slimes not detected on equipment in line. Bins were baffled to minimize damage to fruit.

[g] Plant 3 was a newly constructed plant in its first day of operation at the time the juice of Pack D was prepared.

Experimental Procedure

The initial analysis was made after the juice had been frozen for two weeks. Subsequent analyses were made at various intervals thereafter. The containers were thawed by submersion in running tap water. The cans were briskly shaken to mix the contents and aseptically opened. Samples were removed for plate counts and coliform analysis.

Plate counts were made from suitable dilutions of the juice on tryptone glucose extract agar (pH 7) and on potato dextrose agar (pH 5.6). The plates were counted after they had been incubated for 4 days at 31° C. (87° F.).

The recommendation of Dr. R. V. Stone of the Los Angeles County Health Department (personal communication) that the juice be neutralized with sterile NaOH solution was followed in preparation for coliform analysis. About 16 to 18 cc. of *N* NaOH were found necessary to neutralize 100 cc. of the juice to pH 6.6 to 7.0.

Standard methods of the American Public Health Association *(1)* were followed in determining the coliform index. All cultures which were positive to the completed coliform test were differentiated by the recognized IMViC tests and run through the boric acid medium described by Vaughn and Levine *(15)*. Kovac's test for indol, the Vaughn et al. *(16)* modification of the Voges-Proskauer test, and the Koser citrate test were used.

The term "coliform" as used in this paper refers to any Gram-negative non-sporulating rod which ferments lactose with gas production and ranges from aerobic to facultative in its oxygen requirements. Where specific names are used, the cultures in question have been differentiated and classified as characteristic of *E. coli* or *A. aerogenes* defined in Official Methods of Water Analysis *(2)*. The term "intermediate" refers to those coliform organisms having IMViC characteristics falling between those of *E. coli* and *A. aerogenes*.

Experimental Results

The initial plate counts made on the frozen juice reflected the apparent sanitary conditions in the plants which had produced the juice. Packs B and C were much higher in microbial content than were the other two packs of juice. In all packs the plate counts decreased as the freezing storage periods progressed (Table 2). This progressive decrease appeared to be logarithmic in nature, although in Pack A and to a lesser extent in the other packs, a more rapid drop in count was obeserved during the early storage phase (Figure 1). This rapid initial drop suggests a mixture

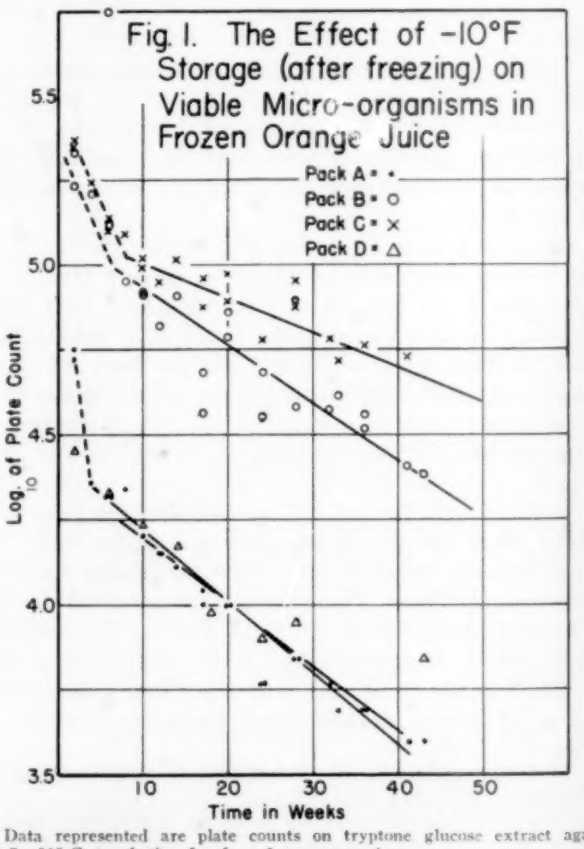
TABLE 2

Plate Counts of Orange Juice Samples Stored at —10° F. (1000's per cc.)

(Plates were counted after 4 days at 31° C.)

Storage Time in Weeks	Pack A		Pack B		Pack C		Pack D	
	TGEA	PDA	TGEA	PDA	TGEA	PDA	TGEA	PDA
2	57	28	172	171	221	160	28	23
	53	22	217	198	225	197		
4	23	11	165	112	175	109		
6	21	13	132	121	137	125	21	15
	21	12	617	702	129	126		
8	22	26	89	83	126	105		
10	16	11	83	62	105	104	17	14
			82	90	97	103		
12	14	11	66	53	90	98		
	14	12						
14	13	9	81	71	105	101	15	15
17	11	8	48	40	91	95		
	10	8	41	43	75	96		
18							9	8
20	10	4	61	48	94	103		
	10	5	72	42	78	105		
24	6	5	48	43	61	74	8	9
	6	5	36	37	60	55		
28	7	4	38	28	75	63	9	9
	7	4	78	63	90	78		
32	6	5	38	34	60	77		
33	5	4	42	41	52	62		
36	5	4	37	45	58	66		
	5	4	33	37	58	70		
41	4	4	31	27	54	68		
43	4	4	24	29			7	7

TGEA = tryptone glucose extract agar, pH 7.0.
PDA = potato dextrose agar, pH 5.6.



Data represented are plate counts on tryptone glucose extract agar, pH 7. 31° C. incubation for four days was used.

TABLE 3

Coliform Index (Most Probable Number per 100 cc.)[b] of Orange Juice Stored at —10° F.

Storage Time in Weeks	Pack A		Pack B		Pack C		Pack D	
	Presumptive	Completed	Presumptive	Completed	Presumptive	Completed	Presumptive	Completed
2	540	11	>1600	39	>1600	14	170	11
	220	5.6	920	46	>1600	48	....	....
4	240	7.8	>1600	6.8	>1600	12	....	....
6	920	46	>1600	6.8	920	7.8	350	13
	240	2	540	26	>1600	7.8	....	....
8	350	0	920	9.2	1600	0	....	....
10	350	7.8	350	4.5	920	14	350	11
			1600	4	1600	7.8	....	....
12	540	2	92	4.5	920	17	....	....
	350	2	....	....	....	....	....	....
14	170	0	>1600	4.5	>1600	7.8	240	7.8
17	220	7.8	350	17	540	4	....	....
18	220	2	350	4	>1600	4.5	29	6.8
20	170	2	1600	4	920	0	....	....
	920	0	920	8.5	1600	4	....	....
24	350	17	920	13	920	2	79	2
	240	4	1600	12	540	9.3	....	....
28	540	17	350	11	920	13	79	0
	350	4.5	920	6.8	240	2	....	....
32	350	2	48	2	240	4	....	....
33	240	0	540	22	540	17	....	....
36	210	9.1	920	6.8	350	23	....	....
	350	4	540	23	540	4.5	....	....
41	350	0	22	0	240	4.5	....	....
43	70	1.8	1600	17	....	....	640	1.8

[b] The data presented are arrived at by comparing the experimental results obtained in the presumptive and the completed coliform tests with Table 17B (most probable numbers) coliform group test, Standard Methods for Water Analysis. 9th Ed., 1946.

of resistant and sensitive cells which, according to Rahn *(11)*, would behave in this manner. This conclusion is confirmed by the reduction in number of different types of colonies on the petri-dish cultures as the storage period continued. At the end of the experiment, most of the colonies on the plates were punctiform and required careful observation to avoid confusion with extraneous matter in the plates.

Except for results with Pack D, the trend in coliform survival as measured by the completed coliform index was not as uniform as were the trends in total numbers of micro-organisms. The variation from sample to sample within Packs A, B, and C was marked and there appeared to be little correlation between the presumptive index and the completed coliform index (Table 3). For example, 17 of the 71 samples in these three packs had a presumptive coliform index of 1600 or more per 100 cc. The completed coliform index of these 17 samples varied from 0 to 48 per 100 cc. and was less than 10 per 100 cc. in 11 of the 17 samples. The data obtained from differentiation of the cultures which were positive to the standard completed coliform test are presented in Table 4.

In all, 236 coliform cultures were isolated from the 79 samples of juice analyzed. If all the tests had been positive on completion, 1,185 coliform cultures would have been isolated. Of the 236 cultures isolated 46, or 19.5 percent, had the + + − − or − + − − IMViC reactions, which would indicate that they resembled *E. Coli;* 20, or 8.5 percent, had the + + − + or − + − + reactions characteristic of the intermediates, and the remaining 170, or 72 percent, were Aerobacter (IMViC − − + + or + − + +).

Coli-like (IMViC + + − − or − + − −) organisms were most frequently present in the two packs prepared from soft fruit (Packs A and C). No definite statements can be made about the role that the fruit played as a source of the coliform bacteria, since studies of the external and internal flora of the oranges were not made at the time the packs of juice were obtained.

Discussion

The results reported above appear to differ greatly from the findings of Schrader and Johnson *(12)*, who state that organisms of the coli type would fail to survive for longer than two weeks in frozen orange juice. All 46 of the coli-like cultures in the present study were isolated after the juice had been frozen for at least two weeks. If Schrader and Johnson based their statement on plate counts, the answer may lie in the fact that the plate counts are not sufficiently sensitive to detect small

TABLE 4

Types of Coliform Organisms Isolated from Samples Stored at —10° F.[1]

—10° F. Storage Time in Weeks	Pack A			Pack B			Pack C			Pack D		
	A	I	C	A	I	C	A	I	C	A	I	C
	Numbers of Each Type Isolated											
2	1	1	2	10	0	0	5	0	0	4	0	0
	0	1	2	7	0	0	8	0	3	..	..	..
4	1	0	2	2	1	0	4	0	1	..	..	..
6	2	1	4	2	1	0	0	3	0	2	2	0
	0	0	1	4	1	1	2	0	1	..	..	..
8	0	0	0	4	0	0	0	0	0	..	..	..
10	1	1	1	2	0	0	4	0	1	4	0	0
				2	0	0	3	1	1	..	..	..
12	0	0	1	1	0	1	2	0	1	..	..	..
	0	0	1									
14	0	0	0	2	0	0	2	1	0	2	1	0
17	1	0	2	5	0	0	2	0	0	..	..	..
18	0	1	0	2	0	0	2	0	0	3	0	0
20	0	0	1	2	0	0	0	0	0	..	..	..
	0	0	0	3	0	0	1	0	1	..	..	..
24	0	0	5	6	0	0	1	0	0	1	0	0
	0	1	1	6	0	0	4	0	0	..	..	..
28	0	0	5	5	0	0	2	0	2	0	0	0
	0	1	1	3	0	0	1	0	0	..	..	..
32	0	0	1	1	0	0	2	0	0	..	..	..
33	0	0	0	6	0	0	5	0	0	..	..	..
36	1	2	2	3	0	0	3	0	2	..	..	..
	1	0	1	5	0	0	2	0	0	..	..	..
41	0	0	0	0	0	0	2	0	0	..	..	..
43	0	0	1	5	0	1	..	..	..	1	0	0

A = Aerobacter spp. (IMViC − − + +). I = Intermediates (IMViC + + − +, − + − +, + + + +). C = *E. coli* (IMViC + + − − or − + − −).

[1] The data represent the classication of the cultures positive to the completed coliform tests.

numbers of bacteria unless large numbers of plates are used. In none of the samples examined in the present studies was the coliform index greater than 1 per 2 cc., and it is quite conceivable that most of the coliforms would have been missed had routine plating been carried out. Other factors which could enter are possible differences in behavior of the organism in pure and in mixed culture and the difference in survival due to different storage temperatures. Prescott et al. *(10)* point out that *E. coli* behaves differently in pure culture from the way it may react when in mixed culture in water analysis. The effect of temperature on the survival of *E. coli* in acid media has been studied by Wallace and Tanner *(18)*. They showed that *E. coli,* when frozen in acid media, would survive longer at very low temperatures than at temperatures nearer the freezing point.

The use of the presumptive test in detecting coliforms in orange juice should not be used as the criterion of fecal contamination, since the sugars present in the inocula may be fermented by yeasts and other inocuous micro-organisms to give false positive tests. This is well borne out by the data in Table 3, in which the presumptive indices were many times those found when the tests were carried to completion. This large difference indicates that the presumptive test cannot be relied upon to give an accurate picture of the actual coliform content of the juice and that the test must be carried to completion if a dependable coliform index is to be obtained. To avoid confusion caused by fermentation of sugar present in some of the beverages by yeasts and not by coliforms, the American Public Health Association *(3)* recommends that evidence of gas obtained when bottled beverages are analyzed must be confirmed by transplanting into differential media for verification before reporting. Since orange juice resembles many of the bottled beverages more than it does drinking water, the same should hold for this product.

Regarding the type of coliforms present, it must be pointed out that not all coliforms should be considered of fecal origin. In fact, the 9th Edition of Standard Methods for the Examination of Water and Sewage *(2)* lists only those organisms having IMViC reactions of + + − − or − + − − as commonly designated of fecal source, while the other members of the group are listed as non-fecal in their commonly designated source. *A. aerogenes* is considered to be normal to plant materials and is often referred to as the "grass or grain strain" of the coliform group of organisms.

An opinion on the significance of coliform types has been expressed by Hunter *(5)*. He brings attention to the fact that *E. coli* is useful as a measure of excretal contamination, but that coliform organisms of the *Aerobacter* genus do not have sufficient sanitary significance to be of definite value as indices of pollution, except in special instances. Hunter also pointed out that the presence of coliforms on raw fruits and vegetables is of doubtful sanitary significance and that the limitations of coliform tests must be recognized. Since frozen orange juice is definitely a raw fruit product, limitations of the standard coliform tests should be recognized and some means for differentiation of the coliforms required.

Another item which may confuse the coliform picture when the organisms are isolated from plant material is the fact that some plant pathogens (members of the genus *Erwinia*) may resemble the coliforms. Elrod *(4)* reported that had a number of *Erwinia* cultures he studied been isolated in the course of a coliform investigation they would most certainly have been classed as aberrant coliforms. Stuart et al. *(13)* also demonstrate marked similarities between some *Erwinia* and the coliform group, reporting that in some cases the strains of phytopathogens could not be distinguished from coliform strains when compared by the usual methods. The *Erwinia* are reported to resemble the intermediates more than *A. aerogenes* or *E. coli (4)*, but Waldee *(17)* has found a plant pathogen, *Pectinobacterium delphinii,* which has the IMViC reactions of *E. coli,* although it differs from *E. coli* in the gelatine and protopectin tests and in the fermentation of a number of carbohydrates. The bacteria isolated during the course of the present study were not routinely run through vegetable maceration tests to check their phytopathogenicity (characteristic of the *Erwinia* group). However, in 27 tests on cultures of each type of coliform found in orange juice none attacked vegetable tissue. Definite statements regarding the sanitary significance of coliform organisms in frozen juice cannot be made at this time. To do so one must know more about the bacterial flora in each step in the handling of the oranges between the grove and the packaged juice. Studies on the effect of handling practices of the fruit on bacterial populations in the juice are contemplated.

Summary

Studies have been made on 79 samples of commercially prepared frozen orange juice. Plate counts and coliform content of the juice were followed periodically over a storage period of 43 weeks at —10° F. (—23° C.).

A rapid drop of total bacterial numbers was observed during the early part of the storage period. Later the drop was less and followed a logarithmic slope. This decline in rate suggests that a mixture of sensitive and resistant micro-organisms was originally present in the juice.

The survival of coliforms in the frozen juice did not follow the trend that was observed with the plate counts; it became less in Pack D as the storage period progressed, but showed little definite trend in the other packs. A definite correlation of magnitude of presumptive coliform index with magnitude of the completed coliform index was not found.

Out of 1185 initial inoculations into lactose broth, a total of 236 coliform cultures were isolated from the 79 samples of juice in the course of the experiment. Forty-six of these cultures resembled *E. coli,* 20 resembled the intermediates, and 170 resembled *Aerobacter* spp.

Organisms resembling *E. coli* were recovered most frequently from the two lots of juice prepared from soft fruit. The pack produced from sound fruit in a clean plant contained none of these organisms.

Coliforms remained viable during the entire experiment in single-strength juice stored at —10° F.

(—23° C.) for 43 weeks and *E. coli*-like organisms (IMViC + + — — or — + — —) were isolated from Pack A and Pack B at the end of this period.

The results apply to the packs of juice studied and should not be interpreted as being representative of all packs of frozen single-strength juice which are marketed.

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Comparative Value of Carbonyl Enediols in Retarding Enzymic Browning of Fruits[a]

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The authors found that cyclic and acyclic carbonyl enediols including l-ascorbic acid, reductic acid, reductone and dihydroxymaleic acid retarded enzymic browning of defrosted peaches, apples, pears and apricots. Sodium and ammonium dihydroxymaleate were ineffective.

In 1939 Gray and Stone reported results of experiments in which l-ascorbic acid, d-gluco ascorbic acid, reductone and dihydroxymaleic acid were used to prevent certain undesirable oxidations in beer *(4)*, and development of rancidity in fat-water emulsions such as mayonnaise *(3, 5)*. The possibility that compounds of this type might find wide application as antioxidants in the food industry was fully recognized by these investigators. Tressler and Du Bois published in 1942 an account of experiments which had extended over six seasons. It was found that l-ascorbic acid, d-iso ascorbic acid and d-gluco ascorbic acid were all effective in retarding the enzymic brown discoloration of the cut surfaces of different fruits, and that this was particularly true for frozen and defrosted fruits. At about the same time Fellers, Esselen, Fitzpatrick, Moore and Powers *(2)* described briefly results of experiments in which l-ascorbic acid, d-iso ascorbic acid and d-gluco ascorbic acid had been added to glass packed fruits and vegetables as antioxidants. Subsequently Yourga, Esselen and Fellers *(9)* found that solutions of d-iso ascorbic acid were oxidized more readily than those of l-ascorbic acid. This led to further work in which Esselen, Powers and Woodward *(1)* showed that, because of its preferential oxidation by molecular oxygen, d-iso ascorbic acid, when added to fruit or vegetable juices, protected l-ascorbic acid from oxidation. In consequence of these various investigations l-ascorbic acid has been widely applied industrially as an antioxidant, especially in the frozen fruit industry.

During a study of the control of oxidative rancidity in frozen fish the comparative antioxidant value of certain cyclic and acyclic carbonyl enediols, which are closely related to l-ascorbic acid in certain of their

[a] Presented before the Ninth Annual I. F. T. Convention, San Francisco, Calif., July 14, 1949.

chemical properties, was determined by the writers. It was found that, while the cyclic compounds were quite effective for this purpose, the acyclic compounds were ineffective, or nearly so. The value of these compounds in retarding enzymic browning of fruits was investigated with the following results.

Experimental

The sources and methods of preparation of the various carbonyl enediols employed have been described elsewhere *(7)*. Sound, ripe fruits purchased on the local retail market were used, the detailed methods of preparation of these being given in the individual experiments. In all instances procedures were carried out very rapidly in order to avoid browning of the fruit before freezing. Approximately 75 g. of prepared fruit were added to each of a number of 6 oz. screw cap glass jars, and the contents then covered with 50 ml. of 25 or 50 percent by volume cold sucrose syrup, with or without added antioxidant. The carbonyl enediol antioxidants were incorporated in the sucrose syrups in the same molecular concentration immediately before adding to the fruits. The amounts added were: l-ascorbic acid, 0.04%; dihydroxymaleic acid, 0.0357%; sodium dihydroxymaleate, 0.0435%; ammonium dihydroxymaleate, 0.0415%; reductic acid, 0.0259% and reductone, 0.02%. The filled jars were frozen in an air blast at —20° C. and stored at this temperature. Representative samples were removed at intervals, thawed at room temperature in an air blast, and the contents then placed in 15 cm. petri dishes. The relative amount of visible brown discoloration was recorded at suitable intervals after thawing. In certain instances the samples were photographed.

Peaches. J. H. Hale peaches were dipped 1 minute in boiling water, cooled promptly in cold water, skinned, sliced and frozen in 50% sucrose syrup. The results obtained on thawing samples after 2 days' storage are recorded in Table 1. They show that reductone, reductic acid and dihydroxymaleic acid were all somewhat more effective than l-ascorbic acid in retarding browning, while the sodium and ammonium salts of dihydroxymaleic acid were practically ineffective. Similar results were obtained with samples defrosted after 140 days' storage (Figure 1).

TABLE 1

Effect of carbonyl enediols on the enzymic browning of defrosted Hale peaches

Treatment	Relative intensity of browning[b] after defrosting			
	4 hr.	6 hr.	24 hr.	48 hr.
Untreated	E	F	F	G
l-Ascorbic acid	C	D	D	E
Dihydroxymaleic acid	B	C	D	D to E
Sodium dihydroxymaleate	D	D to E	E	F to G
Ammonium dihydroxymaleate	C	D	E	F to G
Reductic acid	C	C	C to D	D
Reductone	A	A	C to D	D

[b] A = No appreciable discoloration. B = Faint brown. C = Slight brown. D = Moderate brown. E = Marked brown. F = Dark brown. G = Intense brown to black.

This arbitrary method of recording comparative color changes is used in all tables.

Apples. Thin slices of Winesap apples were immersed 15 minutes in ice cold 4% sodium chloride solution, drained, packed in glass jars and covered with 25% sucrose syrup in the usual manner. Before freezing the jars were exposed to a partial vacuum (1.4 cm. of mercury for 15 minutes) in order to remove dissolved air from the apple cells and to facilitate penetration of the sucrose solutions. The results obtained on thawing samples after 103 days' storage are recorded in Table 2 and in Figure 2. In this experiment dihydroxymaleic acid and l-ascorbic acid proved somewhat more effective than reductone and reductic acid in retarding browning.

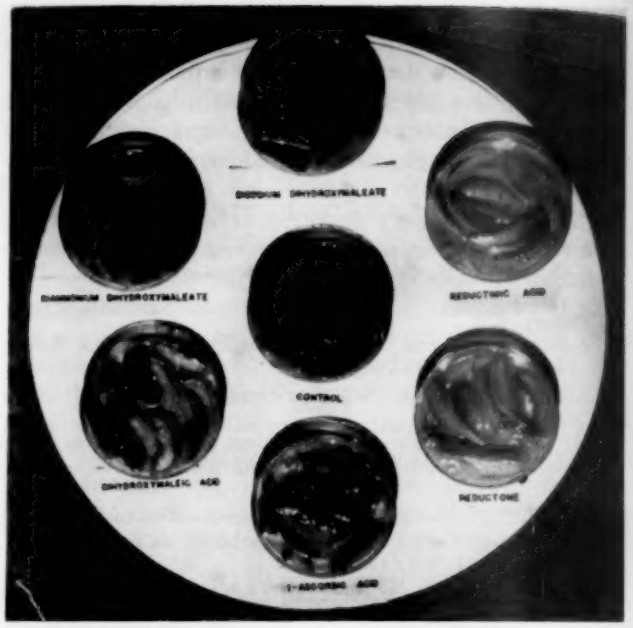

FIG. 1. Peaches. Browning following thawing after 2 days' storage.

TABLE 2

Effect of carbonyl enediols on the enzymic browning of defrosted apples

Treatment	Relative intensity of browning after defrosting				
	4 hr.	5 hr.	7 hr.	24 hr.	48 hr.
Untreated	D	E	E to F	F	F
l-Ascorbic acid	A	A	B	C to D	D
Dihydroxymaleic acid	A	A	B	C	D
Reductone	A	B	C	D	E
Reductic acid	A	B	C	D	E

Apricots. Moorpark apricots were washed in cold water, halved and frozen in 50% sucrose syrup. Samples were thawed after 2 days' storage and the results are given in Table 3. The protective effect of the antioxidants was neither as marked nor as prolonged as in the two foregoing experiments. Dihydroxymaleic acid and reductic acid retarded browning more markedly than did l-ascorbic acid and reductone during the first 6 hours after thawing. However, 24 hours after thawing discoloration was about equally intense in all samples.

Pears. Bartlett pears were skinned, sliced and frozen in 50% sucrose syrup. Samples were thawed after 140

TABLE 3

Effect of carbonyl enediols on the enzymic browning of defrosted apricots

Treatment	Relative intensity of browning after defrosting		
	4 hr.	6 hr.	24 hr.
Untreated	C	E to F	G
l-Ascorbic acid	B	C	G
Dihydroxymaleic acid	A	B	G
Sodium dihydroxymaleate	C	E to F	G
Ammonium dihydroxymaleate	C	E to F	G
Reductic acid	A	B	G
Reductone	B	C	G

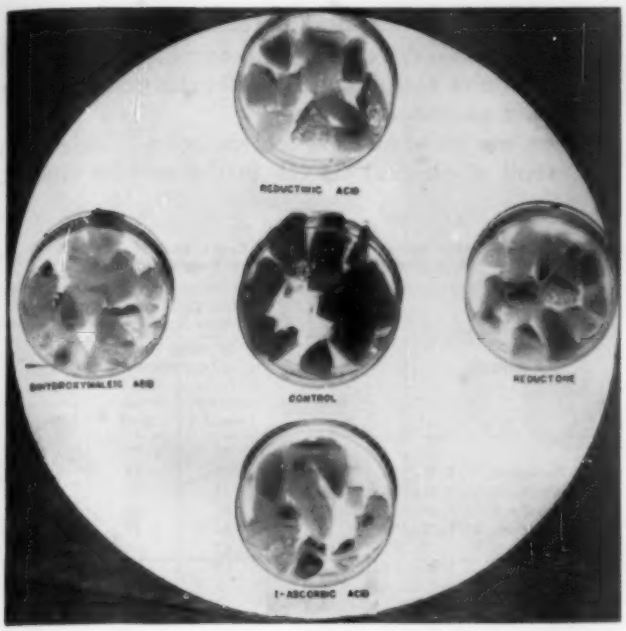

FIG. 2. Apples. Browning following thawing after 103 days' storage.

days' storage. The results showed that dihydroxymaleic acid and l-ascorbic acid afforded greatest protection against browning, reductone and reductic acid proving only slightly effective and the sodium and ammonium salts of dihydroxymaleic acid ineffective.

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Processing Quality of Varieties and Strains of Dry Beans[a]

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A brief review of the literature on hardshell beans is followed by the experimental results on 12 samples representing 4 varieties of beans. The factors studied were rates of water imbibition, percentages of hardshell beans in samples, effect of hot water treatment on hardshell beans, cooking and processing qualities. The effect of different relative humidities on equilibrium moisture content was determined for one sample.

Variations in the permeability of seed coats of many different kinds of plants have been recognized for centuries. Theophrastus *(11)* in the third century B. C. referred to the differences in beans as "cookable" or "uncookable," and he also recognized that certain conditions of climate may cause this variation. He stated that "In the district of Philippi, if the beans while winnowed are caught by the prevailing wind of the country, they become uncookable, having previously been cookable."

Two types of hardness in beans were described by Gloyer *(2)* in 1921. A condition of the cotlyledon whereby this portion of the bean does not imbibe water was called sclerema. Snyder *(10)* also reported encountering some beans that would not imbibe water even though the seed coat was scarified or removed. Gloyer suggested that this condition may be caused by enzymic changes during the storage of seeds in damp atmospheres at high temperatures. This kind of hardness in beans is much less commonly encountered than is the kind of hardness described by Gloyer that is due to hardshell, or impermeable seed coat. This latter condition is fairly common in most legumes, and has been thoroughly studied in connection with seed germination work. The information that has been accumulated by seedmen should prove of value to food processors.

In part, the hardshell character of beans is hereditary *(7)*. By plant breeding and selection, strains have been developed that contain practically no hardshell beans under favorable conditions *(4)*. Unfavorable environmental factors following planting, such as cold rainy weather, are recognized as factors that cause a progeny to contain a higher percentage of hardshell beans than would result if normal conditions prevailed during the germination period *(5)*. The non-hardshell beans that imbibe water readily frequently rot as a result of cold wet soil conditions, whereas the hardshell beans endure the unfavorable period and later germinate. This sort of chance selection may be responsible for some of the differences that occur in the hardshell character of beans from year to year, or between different plantings the same year.

The climatic conditions under which beans are grown probably affect the degree of hardshell present in a given lot of beans at harvest time. Beans grown and

[a] Report of a study made under the Research and Marketing Act of 1946.

[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

harvested in a hot, dry area are believed to be more subject to development of hardshell than are beans grown at lower temperatures, together with higher relative humidities.

Another very important factor that influences the degree of hardshell in some varieties of beans is the storage environment. Gloyer *(3, 5)* has studied the relationship of humidity and temperature to hardshell character of peas and beans. Referring to beans, Gloyer states that "when the seed is stored in a heated room, hardshell soon develops. The lower the relative humidity of the atmosphere, the greater is the tendency toward hardshell."

Gloyer reported *(6)* the percentage of hardshell in 355 varieties of beans as received from seedmen and 242 varieties of peas grown at New York Experiment Station and hand threshed, all stored during the winter in the Laboratory. Seeds not showing any indications of being permeable to water after 24 hours' soaking at room temperature were considered by Gloyer as hardshell. The beans varied from 0 to 80 percent hardshell, and the peas varied from 0 to 82 percent hardshell.

If beans that contain some hardshell are planted, or processed for food, without treatment, a poor stand of plants, or a product of low textural quality, may result. For this reason in the utilization of hardshell beans for seed, or for food purposes, they are generally treated by some method that alters them so that they imbibe water. Treatment of some legume seeds by concentrated sulfuric acid for short intervals to increase the percent germination has been used with success *(8)*. Scarification of the seed coat may be used to increase the rate of water imbibition. This method increases the rate and percent of germination of most kinds of seeds where hardshell is a problem. Scarification is also effective in elimination of hardshell beans that are to be processed for food. This method is not extensively used by bean processors, however, because very careful control would be required in order to eliminate broken seed coats after processing.

A very effective procedure that is both practical and convenient for the food processor consists of steaming the beans or treating them with hot water prior to soaking them. Gloyer *(2, 3)* mentioned this method of eliminating hardshell in beans. Bitting *(1)* also referred to it in his book.

The work of this laboratory in evaluating the processing quality of beans developed for disease resistance by the research staff at Idaho Experiment Station has included six strains of Great Northern, two strains each of pinto and Red Mexican, one of small flat white, and an Idaho-grown Michelite sample. A Michigan-grown Michelite sample was also used. The study on these samples consisted of determining (a) the physical defects (broken beans, cracked seed coats), (b) rate of water imbibition, (c) percentage of hardshell beans after 5 and 24 hours of soaking, (d) effectiveness of hot-water treatment on the elimination of hardshell beans, (e) cooking quality (f) processing quality and (g) for the Michigan Michelites only the equilibrium moisture contents and hardshell percentage at relative humidities ranging from 10 to 100.

Physical Defects

The physical defects were determined by careful examination of each bean in a 500-gram sample under low-power magnification (2x).

There was about an eight-fold variation (Table 1) in the extent of physical defects, in the samples studied.

TABLE 1

Percentages of Beans having Cracked Seed Coats, and of Hardshell Beans Before and After Hot-Water Treatment

Sample	Cracked seed coats	Hardshell Beans by Count [a]		
		Without Heat Treatment		1 Minute Immersion in Boiling Water
		24-hours soak	5-hours soak	5-hrs. soak
	%	%	%	%
Great Northern, U. I. No. 15	8.3	7	35	2
Great Northern, U. I. No. 16	11.1	25	51	3
Great Northern, U. I. No. 31	14.0	38	57	1
Great Northern, U. I. No. 59	14.6	34	45	5
Great Northern, U. I. No. 81	19.3	45	50	4
Great Northern, U. I. No. 123	2.6	19	72	1
Mexican Red, U. I. No. 3	4.0	1	49	2
Mexican Red, U. I. No. 34	6.3	2	66	3
Pinto, U. I. No. 111	5.6	17	58	0
Pinto, U. I. No. 78	14.0	1	37	0
Michelite (Idaho grown)	6.0	7	20	3
Michelite (Michigan grown)	2.4	1	1	0

[a] Determined by count of impermeable beans, assigning 50 percent weight to the partly hydrated beans in calculating percentage of hardshell.

The Michigan-grown Michelites contained the smallest percentage of defects (2.4). The largest percentage of defects (19.3) occurred in the Great Northern (University of Idaho No. 81). The Michelite (Michigan) sample was of good commercial grade and had probably been hand picked, whereas the beans from Idaho nearly all represented experimentally grown lots that were not threshed and worked over in the same manner as are beans grown and harvested for commercial use. This difference in method of handling probably was responsible for a part of the increased amount of broken and cracked beans in the samples from Idaho. The low humidity that prevails in Idaho probably caused the beans to be brittle and more subject to thresher damage. Of course differences between varieties may be just as real as differences due to variations in climatic conditions. The reason for comparing them here is to indicate the nature of the material used in these studies.

Rates of Water Imbibition

The imbibition of water was followed in a special piece of apparatus. It consisted of a 100-ml. graduated cylinder and a 250-ml. Erlenmeyer flask joined by means of a standard-taper glass joint. A removable wire diaphragm was made to fit in the neck of the flask. This diaphragm served to prevent the beans from entering the cylinder when the complete unit was placed with the flask in the top position. The sample of beans (25 g.) was placed in the flask, the wire diaphragm was inserted, about 100 ml. of water was added to the cylinder, and the flask was placed on top of the cylinder. The experiment was started by inverting the apparatus, swirling the beans in the water momentarily, and then allowing the water to drain back into the cylinder. After draining for about a minute the zero time reading of the graduated cylinder was recorded and the water poured back on the beans. At the desired intervals the excess

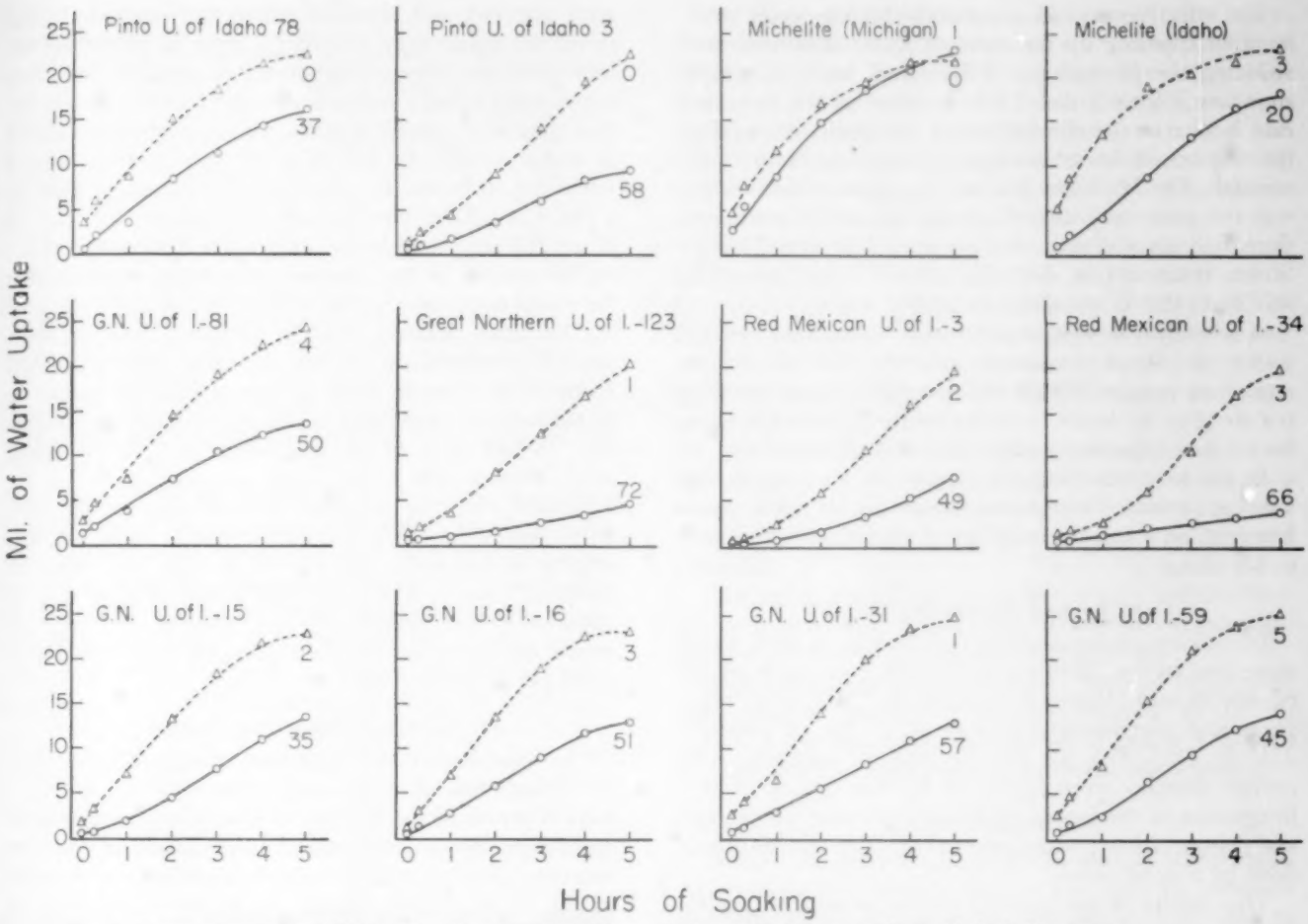

FIG. 1. Rates of water imbibition of various samples of beans before and after one-minute dip in boiling water.

The solid lines represent no treatment and the broken lines represent the hot water treatment. The figures at the end of each curve represent the percentages of hardshell beans after five hours' soaking.

water was measured by pouring it into the cylinder. The method was found to be very simple and at the same time to produce reproducible results. The changes in the character and amount of bean surface with hydration of course influenced the amount of water that drained, but the error due to this factor was small and is inherent in any method that relies on drainage. Since the apparatus was completely closed there was no loss of water by evaporation or spilling. Triplicate determinations were usually made on each sample.

The rates at which the different beans studied imbibed water on soaking at 77° F. (25° C.) are shown graphically in Figure 1. The Michigan Michelites showed the fastest rate of water uptake. The two Red Mexican samples and the Great Northern (University of Idaho No. 123) showed the slowest rates of water imbibition. The second best sample with regard to rate of water uptake was the Idaho-grown Michelites. The difference between the Michelite samples grown in Michigan and Idaho are probably due to different climatic conditions in the growing areas. The effect of soils on bean quality should not be overlooked, however.

Hardshell Beans

Percentages of hardshell beans, determined by soaking: The percentage of hardshell beans was determined by counting the beans that had not imbibed water, based on appearance, after a specified time (Table 1). Any beans that were only partially hydrated were weighed 50 percent in calculating the percentage of hardshell.

In general the Great Northern beans contained more hardshells after 24 hours' soaking than any of the other varieties (7 to 45 percent). The University of Idaho No. 15 contained the smallest percentage of hardshells among strains of Great Northern, after both 5 and 24 hours of soaking. The Red Mexican and pinto varieties after 5 hours' soaking contained many hardshell beans (37 to 66 percent) but after 24 hours' soaking were low in hardshell (1 to 2 percent) except University of Idaho No. 111, which still contained 17 percent hardshell beans. The Michelites after 5 hours' soaking were lower in hardshells than any of the other varieties. The Michigan-grown Michelites were almost free of hardshell beans, whereas the Idaho-grown sample contained 20 and 7 percent, respectively, after 5 and 24 hours' soaking. Differences between the two Michelite samples were also observed in rate of water imbibition.

Effect of moist heat treatment on percentages of hardshell beans and on rate of water imbibition: In a study of effect of hot water on reduction of hardshell, the beans in loose cheesecloth bags were suspended in the water for the desired exposure. After heat treatment the beans were cooled, soaked the desired length of time, and then the percentage of hardshell was determined by count.

The effectiveness of a one-minute boiling-water treatment in speeding up the rates of water imbibition and reducing the percentages of hardshell beans is vividly shown in Figure 1 and Table 1. Most of the increased rate is due to the elimination of hardshell beans; thus the number of beans available to take up water is increased. The Michigan Michelite sample imbibition rate was not materially altered by the hot-water treatment, since it contained only one percent of hardshell beans. Steam treatment at 212° F. (100° C.) had an effect similar to that of treatment in boiling water.

A secondary beneficial effect of a one-minute boiling-water or steam treatment is reduction of micro-organisms present. While this short heat treatment does not sterilize the beans, it undoubtedly reduces the number of live organisms materially and therefore can be a factor in controlling the extent of souring in the soaking period. This treatment can not be relied upon, however, as a substitute for good sanitation subsequent to blanching.

Cooking and Processing Quality

In cooking studies a number of samples of each strain were cooked for different periods of time, such as 50, 65, 80, 95, and 110 minutes at 212° F. (100° C.). Samples were also pressure-cooked at 10 lbs. of pressure 221° F. (115° C.) for 20, 30, and 40 minutes. The cooked samples were appraised for the degree of disintegration of the beans, uniformity of color, percentage and degree of hard beans, and flavor. The appraisal was done by a small panel.

The results of the cooking studies confirmed earlier reports with regard to hardshell beans. Unless the beans are treated prior to soaking, the hardshell beans are still hard after a cook, sufficient to make the non-hardshell beans palatable. Mechanical abrasion, or a heat treatment in either hot water or steam for about one minute, is effective in causing the hardshell beans to imbibe water normally, and thus the method results in a product of more uniform texture when cooked. The prolonged cooking of bean samples until untreated hardshell beans become palatable may result in severe disintegration of some of the cooked product. Considerable variation between varieties exists in the amount of cooking they will tolerate, after becoming palatable, before they disintegrate or mush up. The white varieties (Michelites, Great Northerns, and small flat whites) are more susceptible to mushing due to overcooking than are the Red Mexicans and pintos. These differences in cooking quality have been observed for both the open-kettle method and pressure cooking in open containers.

Studies on the processing behavior of hardshell beans in canning were made with Great Northern, University of Idaho No. 81, Red Mexican, University of Idaho No. 54, and pinto, University of Idaho No. 111. Three methods of treatment were compared. First, the dry beans (both hardshell and non-hardshell) and distilled water were placed in a No. 2 can and then processed immediately without any pretreatment or soaking; second, the beans were soaked 16 hours in distilled water, the hardshell beans were segregated and placed in the No. 2 can separated from the non-hardshell beans by a piece of No. 50 filter paper, and then the contents were covered with distilled water and processed; and third, the beaus were treated the same as in the second case with the exception that the segregated hardshell beans were given a one-minute dip in boiling water before they were placed in cans. The cans were exhausted in water at 210° F. (99° C.) for five minutes, sealed while hot, followed by retorting 70 minutes at 240° F. (116° C.). After standing at room temperature for 24 hours the processed beans were opened for inspection.

The results of the canning experiment indicate that hardshell beans are not a problem in canning. At least the hardshell beans present in the beans used in these studies presented no special problems. The hardshell beans in all three varieties cooked sufficiently tender to be palatable even without soaking previously to processing. In fact most of the beans appeared to be cooked more than would be required for palatability by the minimum retorting required for adequate sterilization. After processing, the hardshell beans were slightly smaller in size and a little darker in color than the non-hardshell ones. The color difference would not be apparent in the presence of sauce, and the size variation would hardly be noticed in beans that were not segregated before processing.

Equilibrium Moisture Contents

The equilibrium moisture contents at different relative humidities at room temperature 77° F. (25° C.) were determined by storing 50-gram samples of Michigan Michelite beans in desiccators with the required concentrations of sulfuric acid to give relative humidities of 10, 20, 30, 40, 50, 60, 70, 80, 90, 94, 96, and 98, and over water to give a value of 100. The samples were weighed weekly at first, and later at long intervals until they had reached constant weight. Their moisture contents were determined by a vacuum oven method *(9)*; using 40 hours at 167° F. (70° C.), at less than 3 mm. Hg pressure.

The moisture content of the Michigan Michelite beans was about 14 percent at the time they were placed in the desiccator at the different relative humidities. The rate of change in moisture content was proportional to the relative humidities at the value of 70 percent and above. At values of 60 percent and below the rate of change initially was inversely proportional to the humidities. As one would expect, as the equilibrium values were approached the rate of change became slower unless the moisture content rose sufficiently to support the growth of micro-organisms. The equilibration was a slow process. About five months were required for final moisture equilibrium in the lower humidity range. The samples were stored at their respective humidities for an additional six months before their moisture contents were determined. The equilibrium moisture values ranged from 8.7 to 15.2 percent for relative humidities 10 and 70 percent, respectively (Table 2). The moisture contents of samples at relative humidities above 70 percent were not determined, because mold growth occurred before they reached moisture equilibrium. The moisture contents of the beans at the time mold growths were first observed were estimated by the gains in weights of samples up to the time the mold appeared. These moisture values (Table 2) ranged from 20 percent to 24 percent for

TABLE 2

Equilibrium Moisture Values for Michelite Beans and Relationship of Storage Relative Humidity to Appearance of Mold

Relative Humidity	Equilibrium Moisture	Moisture Content [4] When Mold First Noticed	Days Before Mold Appeared
%	%	%	
10	8.7	----	----
20	9.4	----	----
30	10.0	----	----
40	10.4	----	----
50	11.8	----	----
60	13.0	----	----
70	15.2	----	----
80	Mold growth occurred	20.0	136
90	before moisture	22.0	80
94	equilibrium was	23.2	80
96	established	23.0	66
98		23.0	59
100		24.0	32

[4] Estimated on basis of weight gain of sample before mold growth was observed.

samples stored at 80 and 100 percent relative humidity, respectively. The days of storage before mold growths were first observed likewise ranged from 32 days at 100 percent relative humidity to 136 days at 80 percent relative humidity. The appearance of the beans after storage at relative humidities of 70, 80, and 90 percent for 10 months is shown in Figure 2. The mold colonies on the samples stored at 80 and 90 were quite apparent. It was also observed (Figure 2) that the heaviest growth, and frequently the only growth on a bean in the early stages of growth was limited to the micropile or hilum area. The reason why this area was the most favorable for growth was probably the higher local moisture content and more available nutrients.

FIG. 2. Condition of Michelite beans after 10 months of storage at 77° F. (25° C.) in relative humidities indicated by the number on the picture.

The storage of the Michigan Michelite samples at the different relative humidities of 70 percent and below did not alter their water-absorbing properties materially. The lower the humidity of storage, the slower the initial rate of water inbibition for the first 30 minutes of soaking, but thereafter the rates were about the same, and the total water uptake was as large in the samples that had been stored at low humidities as at higher humidities.

After 11 months of storage in the different humidities there were about 5 percent hardshell beans in the different samples, after soaking five hours, but there was no consistent trend that correlated with the humidity of storage. No hardshell beans were present in the samples after 24 hours' soaking, except the one that had been stored at 10 percent relative humidity, and even in this case less than 2 percent were present.

Summary and Conclusions

Some of the earlier published work on the nature of beans with respect to hardshell character has been reviewed, and methods for treatment of hardshell beans mentioned. A practical method used for processing hardshell beans was a short heat treatment in either hot water or steam.

The rates of water imbibition before and after treatment in hot water are given for six strains of Great Northern beans, two strains of Red Mexicans, two strains of pintos, one strain of small white flat, and two samples of Michelite (one Michigan-grown and the other Idaho-grown). The hot-water treatment greatly accelerated the rate of water uptake and reduced the percentage of hardshell beans markedly.

Cooking soaked samples that contain some hardshell beans until the latter are palatable may require such a long cook that the non-hardshell beans disintegrate. In the present study Great Northern, Michelites, and small white flat proved more subject to disintegration on "overcooking" than did the Red Mexicans and pinto varieties.

The kind of hardshell beans encountered in this study does not require any special treatment for canning purposes, since the minimum retorting necessary for adequate sterilization is a sufficient cook to make them palatable.

Storing Michelite beans at 80 percent relative humidity for 136 days caused them to increase in moisture content sufficiently to support mold growth.

Michigan Michelite beans stored from 10 to 70 percent relative humidities for 11 months at 77° F. (25° C.) did not materially change in water rehydration character or percentage of hardshell beans present.

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Comparative Evaluation of Spices. II. Flavor Evaluation of Natural Spices and Spice Substitutes in Representative Test Foods[a, b, c]

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Natural spices, including capsicums, caraway, cassia, cloves, coriander, ginger, mace, mustard, nutmeg, paprika, pepper, both black and white, and sage, were evaluated in comparison with spice substitutes—spice oils, oleoresins, spice extractives added to salt and sugar—using the organoleptic panel technic. Flavor, including odor, taste and aftertaste, along with color, composed the factors determined. Spices were mixed with dry salt and sugar for comparison with the materials composed of extractives sorbed on salt and sugar. Test foods, including white sauce, oil white sauce, representative foods such as pumpkin, eggnog, and cream sauce, were developed, proving to be useful aids in evaluation.

Analyses of panel scores show that in general spice substitutes were of poorer quality in odor and taste than natural spices. Using increased amounts of the spice substitutes in the evaluation did not increase their quality rating. Separation of flavor-bearing components of natural spices from their original loci appears to result in alteration and reduced acceptability of the spice flavor.

Spices contribute greatly to the flavor of food by providing the completeness of flavor needed to lift it from an unattractive and unpalatable level to one of appetizing desirability. An organized research program was undertaken in order to determine the attributes of natural spices, in terms of flavor, odor, stability, and usefulness, in comparison with various spice-flavoring materials.

Although much is known concerning the chemical nature of spices and of their essential oils, very little has been reported concerning spice flavors and their stability, their similarities and the differences between types of spice flavors. The results obtained throw light on many aspects of food technology such as flavor usages, stability of spice-flavored foods, and the use of spices in preserved foods.

The usefulness of flavor testing and quality evaluation of foods by an organoleptic panel has been demonstrated (1) by application of a technique developed in our laboratories (2). This method has yielded reliable and reproducible results. A less comprehensive test method reported in the literature (3) depends on evaluation of spice aroma from a cold-water infusion. With our method, quantitative values for the flavor and appearance of spiced food samples are reported.

The following types of spices and spice materials were studied: (a) Natural dry ground spices; (b) pure spice oils and oleoresins; (c) spice oils and oleoresins with added emulsifying agents; and (d) extractives from spices blended with sugar or salt as a base.

Method of Evaluation

Before evaluation of a specific sample in comparison with other samples of the same spice, the members of the panel discuss the material under study and agree on a basis for judging flavor. For example, standards of excellence for the fourteen spices studied here were discussed, based on descriptions in standard reference books as follows *(4, 5, 6, 7)*:

Capsicums, Hot: The odor of this spice is distinctive and not unpleasant. The taste is very pungent, sharp, hot and biting. It is difficult to detect more than the hot, burning sensation when placing a small grain on the tongue. The color is a very bright, even red.

Capsicums, Mild: This spice is very similar to the hot variety in odor. However, the flavor sensation is not as strong and biting. Also, as in all capsicums, the color should be a very bright, even red.

Caraway: This spice is quite aromatic, the odor pleasant and penetrating. The taste is pleasant, warm and slightly sharp. The color considered most desirable is a greenish nut brown, varying from light to dark tones.

Cassia: This spice has an agreeable, aromatic odor and a sweet, pungent taste. The color desired is an even, very rich, reddish brown.

Cloves: A very strong aromatic odor and a powerful, cooling, yet pungent, aromatic taste are characteristic of this spice. The color should be a rich, even, dark brown.

Coriander: This spice has a slight, fragrant odor and a pleasant, lemon-y, aromatic taste. The color is variegated yellowish brown.

Ginger: An agreeable, aromatic, somewhat pungent odor and an aromatic, pungent, biting taste are characteristic of this spice. The preferred color is an even, light buff.

Mace: Mace has a mild, pleasant aroma and an aromatic, slightly warm and stimulating taste. The desirable color is light yellow to orange.

Mustard: When made into a water-paste, the odor is sharp, piercing and irritating. The taste is very pungent. The color of mustard flour should be an even, bright yellow.

Nutmeg: This spice has a very characteristic and strongly aromatic odor, and an aromatic, warm, slightly bitter taste. The color should be from very dark brown to light buff.

Paprika: This spice has a mild, pleasant odor and an agreeable, slightly sweet and very mildly pungent taste. The color is a bright rich red.

White Pepper: This spice has a characteristic, penetrating, aromatic odor and a hot, biting and pungent taste, less biting than black pepper. The color should be light gray, with a tan shade in the lighter components.

Black Pepper: This spice has a characteristic, penetrating odor and a hot, biting and very pungent taste. The color should be variegated from the yellowish white particles to dark brown and black particles.

Sage: The odor of this spice is strong, fragrant and aromatic. The taste is aromatic, warm, somewhat astringent, and a little bitter. The color is a rich grayish green.

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[a] Presented at the General Session of the Division of Agricultural and Food Chemistry of the American Chemical Society, at San Francisco, California, March 28, 1949, under the title "A Study of the Physical Attributes of Spices and Spice Substitutes."

[b] Supported by the American Spice Trade Association, Inc., 82 Wall Street, New York 5, N. Y.

[c] The first paper of this series was presented before the Eighth Annual I. F. T. Convention, Philadelphia, Pa., June 8, 1948, under the title "Comparative Evaluation of Spices."

Flavor components were then analyzed and scored in conformity with the following maximum value for each factor:

Factors	Maximum Score
Flavor:	
Odor Strength	25
Odor Quality	25
Taste Strength	20
Taste Quality	20
Aftertaste Quality	5
Aftertaste Duration	5
Total	100

Intensity of odor or taste, whether good or bad, is the criterion for the strength components. Quality of odor, taste and aftertaste is measured by its desirability and acceptability. Aftertaste duration is the degree of lingering, continued recognition.

Extractives on Sugar or Salt Base Compared with Natural Spices Mixed with the Same Base

Comparison was made between commercial sugar- and salt-based spice extractives and natural spices mixed with a calculated quantity of sugar or salt. These commercial products are prepared by separating the flavor ingredients from spices by extraction with solvents, pressure or other processing and mixing the separated extractives with the base. In effect, the extractives are sorbed on the solid particles of sugar or salt. In order to prepare corresponding products which would incorporate natural spices, mixtures were made of individual natural ground spices with sugar or salt in such proportions that the spice flavor strengths in these mixtures would be equivalent to those in the corresponding extractive products. The manufacturers' ratings of the equivalent spice values for their extractives were used for this purpose. For example, ginger had the following equivalent values:

Form of Spice	Equivalent Value by Wt.
Natural ground ginger	100
Ginger extractive on sugar base	50
Oil of ginger	5
Oleoresin of ginger	10
Oil of ginger with emulsifying agent	5

In other words, according to the manufacturers, five pounds of oil of ginger can be used in place of 100 pounds of ground ginger or 50 pounds of ginger extractive on a sugar base.

The results obtained by comparison of commercial sugar- or salt-based spice extractives with natural ground spices mixed with sugar or salt are given in Table 1. Most of the natural ground spices were blends made up of equal parts of each of a number of samples. For example, the blend of hot imported capsicums is composed of three imported samples from different countries. Black pepper blend is composed of equal amounts of Lampong and Malabar black pepper. The samples of natural spices used were representative of those available from commercial production.

TABLE 1

Evaluation of Natural Spices in Comparison with Spice Extractives on Sugar or Salt Base

Spice	Flavor Scores		Comments on Extractives in Comparison with Natural Spices
	Natural Spice Added to Sugar or Salt	Extractives on Sugar or Salt Base	
Capsicums (Red pepper)	90	73 ± 1.9	Rancid, weak [4]
Caraway	90	84 ± 1.6	Odor slightly stronger
Cassia	90	78 ± 2.9	Odor "unnatural, glue-like"
Cloves	90	73 ± 4.4	Odor weaker, slightly rancid
Coriander	90	88 ± 1.0	Odor slightly stronger
Ginger	90	71 ± 2.2	Odor slightly weaker
Mace	90	79 ± 3.1	Odor undesirable, "medicinal"
Nutmeg	90	69 ± 2.4	Odor "oil", very "medicinal"
Pepper, Black	90	76 ± 3.4	Odor slightly sour [4]
Sage	90	78 ± 1.5	Odor "medicinal"

[4] Salt Base.

The natural spices were all given a standard score of 90 on the basis of 100 as a maximum. This makes possible a higher figure for comparative values for other samples, if the samples should have a higher spice quality than that of the natural spice. The flavor scores given in the table are the means of the total scores assigned each test specimen by the four members of the scoring panel. They are followed in each case by the standard error of the mean, indicating the reliability of the results. The scores in Table 1 show that the extractives on salt and sugar bases did not contain the good qualities of flavor normally found in natural spices, having lost some of the natural flavor components in extraction. Odor changes were very noticeable. Capsicums, cloves, and pepper preparations exhibited varying degrees of rancidity.

It was not feasible to evaluate color comparatively, in this case, as the commercial extractive mixtures did not approximate the natural spices in color. Except for cassia and ginger, the extractive mixtures were very light or colorless. The cassia extractive mixture had a reddish brown color quite unlike the "cinnamon brown" of natural cassia.

Spice Substitutes Compared with Natural Spices in Test Foods

In an aqueous medium. An aqueous white sauce made according to our usual formula *(2)* was used for the comparative evaluation of,

natural spices,
extractives on a sugar or salt base,
spice oils,
spice oleoresins,
spice oils mixed with an emulsifying agent,
spice oleoresins mixed with an emulsifying agent.

All of these were samples of commercial products. The oils and oleoresins were manufactured according to the usual procedures. The levels of spice substitutes used were based on the equivalent quantities recommended by the manufacturers. The spice and spice substitutes were incorporated in the white sauce, shaken for 1 hour at 75° C., and evaluated while still warm. Mustard and ginger, in the natural dry spice form, were mixed with a portion of the water from the test foods to allow for enzyme action required to release the flavor components. These suspensions, after standing for 1 hour, were added to the test food.

In evaluation of the available substitutes for each of the natural spices included in this study, our previously described panel scoring method *(2)* was used. The aqueous white sauce containing a predetermined standard proportion of natural spice was used as a known standard, with each component of the flavor score arbitrarily assigned 90 percent of its maximum value, and the white sauces containing "equivalent" proportions (based on manufacturers' stated spice equivalents) of each of the substitutes for this spice were scored against the natural spice standard by a four-member panel. Mean total scores, with their respective standard errors, resulting from these evaluations are given in Table 2.

The scores in Table 2 indicate that all of the substitutes examined were inferior in odor and flavor, when used according to manufacturers' directions, to the natural spices, in an aqueous medium. In two instances, comments by panel members show weakness of flavor in the substitutes, but examination of component scores in the original data shows weakness and inferior quality of odor and flavor for most substitutes. In two instances, rancidity of substitutes was commented upon by panel members, and, in the case of black pepper, the flavor of the emulsifying agent was detected in both oil and oleoresin with added emulsifying agent.

These conclusions are based on the testing of all the available types of these substitutes for natural spices. None has the high level of good quality of odor and flavor of natural spices.

Recognizing the possibility of subjective bias on the part of panel members in scoring test foods of known identity, a method was devised to mask the identity of the natural spices and spice substitutes in the test foods, after the panel members had acquired adequate experience in scoring these foods with known standards. Since practically all of the natural spices were readily identified by their color after addition to the aqueous white sauce, portions of each of a selected group were extracted with ether, acetone, and/or water to remove essential oils and other flavor factors, thus providing odorless and tasteless bases for blending with the substitutes to give the color and appearance of natural spices when added to the test food. In certain cases, e.g. paprika, where color was partly removed by extraction, an approved food dye was added to make the substitute-spiced food match the color of the natural-spiced food. All spiced food samples were then coded to conceal their identities from the panel members during scoring.

In the attempted extraction of all flavor and odor components, difficulties were experienced in obtaining a completely tasteless base. In the case of cassia, for example, extremely harsh and radical treatment would have to be used in order to remove all the flavor components, a method practically destroying the base. A slight sweet flavor remained. This base was used as no other method of masking the cassia oils was available at the time. The extreme treatment, such as we used to obtain these tasteless bases, would easily destroy much of the good flavor and odor quality of the extracted material. This may account for the lower scores obtained in the evaluation of the extracted materials.

The mean total scores, with standard errors, resulting from this series of evaluations are given in Table 3, together with the corresponding mean scores from Table 2 (identity of natural-spiced samples known), and with scores obtained with masked natural-spice standards adjusted to the uniform basis of a total score of 90 for each natural spice.

The results in Table 3 indicate that, while bias on the part of panel members may possibly have resulted in somewhat lower scores for substitute-spiced samples in some cases when the identities of the samples were known, such effect was generally not very significant. In fact, in a number of instances, the substitute-spiced samples received even lower scores when the identities were unknown during scoring. In no case did masking of the identities result in a mean score for a substitute higher than for the corresponding natural spice.

In order to determine if greater amounts of the spice substitutes, higher levels than those specified by the manufacturers as equivalents to natural spice, would result in comparative evaluation scores closer to or equal to those of natural spice, elevated levels of the spice substitutes were compared with natural cassia, ginger, nutmeg and black pepper, in plain white sauce.

The elevated levels of the substitutes to be used were

TABLE 2

Evaluation of Spices and Spice Substitutes in an Aqueous Medium (Plain White Sauce)

Spice	Flavor Scores						Comments
	Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents	
Capsicums, hot	90	74 ± 3.5	None	74 ± 1.9	None	68 ± 2.7	None
Capsicums, mild	90	None	None	73 ± 3.0	None	None	None
Caraway	90	70 ± 4.6	82 ± 2.1	None	74 ± 5.1	None	None
Cassia	90	64 ± 7.2	59 ± 4.9	None	56 ± 3.2	None	Oils lack sweetness
Cloves	90	65 ± 4.6	75 ± 5.3	None	70 ± 6.7	None	None
Coriander	90	79 ± 3.5	77 ± 4.4	None	69 ± 2.3	None	None
Ginger	90	66 ± 2.3	87 ± 3.9	None	63 ± 1.2	69 ± 1.9	Substitutes weak in flavor
Mace	90	70 ± 6.5	80 ± 3.0	None	68 ± 2.1	None	None
Mustard	90	None	70 ± 6.6	None	74 ± 5.8	None	Oils with emulsf'g agent sl. rancid
Nutmeg	90	54 ± 8.5	80 ± 2.5	None	72 ± 3.0	None	None
Paprika	90	None	None	81 ± 3.3	None	None	None
Pepper, white	90	81 ± 1.7	None	None	79 ± 2.9	None	Salt-based extractive sl. rancid
Pepper, black	90	74 ± 2.6	70 ± 2.5	67 ± 5.0	75 ± 3.3	74 ± 2.4	Emulsf'g agent flavor noticeable in oil and oleoresin
Sage	90	75 ± 2.5	76 ± 2.3	None	78 ± 4.0	None	None
Mean	90	70 ± 2.3	77 ± 2.6	75 ± 1.8	69 ± 2.1	74 ± 1.6	None

TABLE 3

Evaluation of Certain Selected Spices and Spice Substitutes in an Aqueous Medium (Plain White Sauce) Showing Comparison Between "Standard Known" Scoring and "Blindfold" Scoring

Spice	Evaluation Method	Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents
Cassia	Standard known	90	64 ± 7.2	59 ± 4.9	None	56 ± 3.2	None
	Standard unknown	87 ± 2.0	71 ± 3.6	80 ± 2.7	None	77 ± 1.4	None
	Adjusted to basis of 90	90	73	83	None	80	None
Caraway	Standard known	90	70 ± 4.6	82 ± 2.1	None	74 ± 5.1	None
	Standard unknown	88 ± 1.9	70 ± 4.6	82 ± 2.1	None	74 ± 5.1	None
	Adjusted to basis of 90	90	72	84	None	76	None
Ginger	Standard known	90	66 ± 2.3	87 ± 3.9	None	63 ± 1.2	69 ± 1.9
	Standard unknown	86 ± 1.6	66 ± 5.7	85 ± 1.2	None	79 ± 1.9	67 ± 5.6
	Adjusted to basis of 90	90	69	89	None	83	70
Nutmeg	Standard known	90	54 ± 8.5	80 ± 2.5	None	72 ± 3.0	None
	Standard unknown	84 ± 3.2	59 ± 1.6	82 ± 2.3	None	67 ± 4.9	None
	Adjusted to basis of 90	90	63	88	None	72	None
Pepper, Black	Standard known	90	74 ± 2.6	70 ± 2.5	67 ± 5.0	75 ± 3.3	74 ± 2.4
	Standard unknown	90 ± 0.6	74 ± 2.6	70 ± 2.5	67 ± 5.0	75 ± 3.3	74 ± 2.4
	Adjusted to basis of 90	90	74	70	67	75	74
Mean	Standard known	90	66 ± 3.4	79 ± 5.1	70	65 ± 3.2	77 ± 7.5
	Standard unknown	87 ± 1.0	68 ± 2.6	80 ± 2.6	67	74 ± 2.0	71 ± 4.5
	Adjusted to basis of 90	90	70	83	69	77	72

TABLE 4

Evaluation of Spices and Spice Substitutes in An Aqueous Medium (Plain White Sauce) Using Elevated Levels of Spice Substitutes Greater Than Those Recommended by the Manufacturers

Spice	Flavor Scores, Using Elevated Levels of Spice Substitutes					
	Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents
Cassia, substitutes increased to		166%	120%	....	210%	....
Scores	90	78 ± 1.1	80 ± 1.6	None	74 ± 2.6	None
Ginger, substitutes increased to		200%	110%	....	150%	150%
Scores	90	68 ± 2.9	79 ± 1.9	None	77 ± 3.1	77 ± 2.9
Nutmeg, substitutes increased to		167%	120%	....	210%	....
Scores	90	74 ± 1.3	78 ± 2.7	None	75 ± 2.7	None
Black Papper, substitutes increased to		113%	125%	117%	125%	115%
Scores	90	76 ± 2.1	78 ± 1.9	75 ± 2.6	76 ± 2.5	71 ± 1.4

determined by a ratio of the total flavor and odor strength scores for each to 45, the strength score of the natural spice or the standard. For example, the mean strength score for the cassia oil, when scored using manufacturer's recommended levels, was 27. In ratio to 45, it was indicated that the amount of the cassia oil used was to be increased a little over two times, to 210 percent.

The scores obtained from the evaluation using these increased levels of the spice substitutes are given in Table 4. Raising the level of spice substitutes brought about slightly higher scores due to an increase in the strength component. In no case was the quality of flavor or odor sufficient to cause the substitute to have a score close to the score of the natural spice. Increasing the levels of the spice substitutes above the amount recommended by the manufacturers results in no appreciable increase in evaluation scores.

In an oily medium. Using an oil white sauce prepared according to our usual formula *(2)*, the same types of spices and spice products tested in plain aqueous white sauce (Table 2) were evaluated for odor and flavor strength and quality. The results obtained when the natural spice samples were used as known standards, with an assigned total score of 90, are given in Table 5. As with the plain white sauce, certain spices were rescored in the oil white sauce by the "blindfold" technique, using the method as previously described. These results are given in Table 6.

Again, in this test food, all spice substitutes examined appear to be less satisfactory in overall quality than the corresponding natural spices. Lack of significant bias in favor of the natural spices by panel members is again indicated by the fact that the relative scores for substitute-spiced foods were not appreciably higher, as shown in Table 6, when identities were masked than when when identities were masked than when identities of the natural spices were known during scoring. In fact, in several cases they were even lower.

Among the substitutes examined, the oils appear to be generally most satisfactory, with oleoresins scoring nearly as well in this particular test food. Averaging the results for each of the other types of substitutes for all of the spices in this series for which they were available, they appear somewhat inferior to the oils and oleoresins, but do not differ appreciably among themselves.

As previously, the evaluation using increased levels of spice substitutes shows that none has total scores greater than the score for the natural spice. Table 7

shows the scores obtained when higher levels of the spice substitutes were used.

In representative test foods. The same spices and spice substitutes were evaluated for odor and flavor in one or another of the group of test foods consisting of pumpkin, cream sauce and eggnog prepared by our usual methods *(2)*. The spiced cream sauce and pumpkin were mixed at 75° C. and submitted to the panel for odor and flavor evaluation while still warm. The eggnog was mixed and scored cold.

The results obtained with the identities of the natural-spiced samples known are given in Table 8, while those obtained with identities masked are given in Table 9. These results show general agreement with those obtained when the spices were scored in either aqueous white sauce or oil white sauce.

TABLE 5

Evaluation of Spices and Spice Substitutes in an Oily Medium (Oil White Sauce)

Spice	Flavor Scores					
	Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents
Capsicums, hot	90	69 ± 2.7	None	79 ± 3.2	None	75 ± 1.6
Capsicums, mild	90	None	None	77 ± 2.3	None	None
Caraway	90	66 ± 5.7	82 ± 2.2	None	82 ± 6.7	None
Cassia	90	64 ± 4.1	74 ± 4.9	None	64 ± 5.5	None
Cloves	90	68 ± 2.1	62 ± 4.5	None	71 ± 5.5	None
Coriander	90	84 ± 2.6	84 ± 2.6	None	60 ± 3.7	None
Ginger	90	55 ± 2.0	74 ± 2.6	None	60 ± 4.2	57 ± 3.2
Mace	90	71 ± 1.7	86 ± 5.5	None	76 ± 6.1	None
Mustard	90	None	70 ± 3.9	None	77 ± 5.1	None
Nutmeg	90	73 ± 5.3	89 ± 0.8	None	66 ± 3.3	None
Paprika	90	None	None	80 ± 5.9	None	None
Pepper, white	90	77 ± 2.1	None	None	84 ± 3.4	None
Pepper, black	90	69 ± 5.7	75 ± 7.6	75 ± 5.5	67 ± 9.8	77 ± 7.5
Sage	90	67 ± 4.6	81 ± 1.2	None	83 ± 2.7	None
Mean	90	70 ± 2.1	78 ± 2.5	77 ± 1.2	72 ± 2.7	70 ± 6.3

TABLE 6

Evaluation of Certain Selected Spices and Spice Substitutes in an Oily Medium (Oil White Sauce) Showing Comparison Between "Standard Known" Scoring and "Blindfold" Scoring

Spice	Evaluation Method	Flavor Scores					
		Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents
Cassia	Standard known	90	64 ± 4.1	74 ± 4.9	None	64 ± 5.5	None
	Standard unknown	90	71 ± 3.6	74 ± 0.4	None	75 ± 1.1	None
	Adjusted to basis of 90	90	71	74	None	75	None
Cloves	Standard known	90	68 ± 2.1	62 ± 4.5	None	71 ± 5.5	None
	Standard unknown	89 ± 1.0	70 ± 2.5	74 ± 4.8	None	73 ± 6.2	None
	Adjusted to basis of 90	90	71	75	None	74	None
Nutmeg	Standard known	90	73 ± 5.3	89 ± 0.8	None	66 ± 3.3	None
	Standard unknown	89 ± 1.0	70 ± 2.4	78 ± 1.9	None	74 ± 0.8	None
	Adjusted to basis of 90	90	71	79	None	75	None
Pepper, black	Standard known	90	69 ± 5.7	75 ± 7.6	75 ± 5.5	67 ± 9.8	77 ± 7.5
	Standard unknown	88 ± 2.0	67 ± 6.5	85 ± 3.0	76 ± 4.7	82 ± 4.7	70 ± 5.1
	Adjusted to basis of 90	90	69	87	78	84	72
Mean	Standard known	90	69 ± 1.9	75 ± 5.5	75	67 ± 1.5	77
	Standard unknown	89 ± 0.4	70 ± 0.9	78 ± 2.6	76	76 ± 2.0	70
	Adjusted to basis of 90	90	71	79	78	77	72

TABLE 7

Evaluation of Spices and Spice Substitutes in an Oily Medium (Oil White Sauce) Using Elevated Levels of Spice Substitutes Greater Than Those Recommended by the Manufacturers

Spice	Flavor Scores, Using Elevated Levels of Spice Substitutes					
	Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents
Cassia, substitutes increased to		140%	140%	---	120%	---
Scores	90	78 ± 2.6	74 ± 2.5	None	74 ± 2.5	None
Cloves, substitutes increased to		150%	160%	---	150%	---
Scores	90	72 ± 2.4	75 ± 2.5	None	77 ± 2.1	None
Ginger, substitutes increased to		130%	100%	---	110%	130%
Scores	90	78 ± 3.1	79 ± 1.0	None	77 ± 1.6	79 ± 2.4
Black Pepper, substitutes increased to		120%	167%	167%	150%	150%
Scores	90	77 ± 2.4	81 ± 1.9	85 ± 2.1	79 ± 1.1	75 ± 2.6

TABLE 8

Evaluation of Spices and Spice Substitutes in Representative Test Foods

Spice	Test Food	Flavor Scores					
		Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents
Capsicums, hot	Cream Sauce	90	77 ± 4.5 [e]	None	81 ± 4.5	None	70 ± 4.4
Capsicums, mild	Cream Sauce	90	None	None	87 ± 0.9	None	None
Caraway	Cream Sauce	90	73 ± 2.1 [e]	81 ± 1.5	None	73 ± 3.9	None
Cassia	Pumpkin	90	79 ± 0.4 [f]	76 ± 2.3	None	65 ± 2.6	None
Cloves	Pumpkin	90	68 ± 1.5	72 ± 2.0	None	63 ± 1.8 [g]	None
Coriander	Cream Sauce	90	76 ± 3.8 [f]	71 ± 4.0 [h]	None	72 ± 2.0 [g]	None
Ginger	Pumpkin	90	62 ± 6.1	87 ± 3.0	None	74 ± 1.4	68 ± 3.5
Mace	Eggnog	90	65 ± 1.3	88 ± 0.9	None	70 ± 2.0	None
Mustard	Cream Sauce	90	None	73 ± 4.3	None	71 ± 6.2	None
Nutmeg	Eggnog	90	66 ± 5.9	81 ± 4.9	None	71 ± 6.3	None
Paprika, domestic	Cream Sauce	90	None	None	79 ± 1.2	None	None
Pepper, white	Cream Sauce	90	76 ± 4.6	None	None	76 ± 4.4	None
Pepper, black	Cream Sauce	90	71 ± 3.3	86 ± 1.9 [g]	87 ± 1.5	85 ± 1.2 [g]	78 ± 7.0
Sage	Cream Sauce	90	60 ± 2.4	83 ± 4.3	None	83 ± 4.9	None
Mean of all scores	....	....	70 ± 1.9	80 ± 2.0	84 ± 2.1	73 ± 2.1	72 ± 3.0

Comments expressed during evaluation: [e] Salty. [f] Sweet taste is noticed. [g] Off-flavor noticed. [h] Slightly rancid.

TABLE 9

Evaluation of Certain Selected Spices and Spice Substitutes in Representative Foods Showing Comparison Between "Standard Known" Scoring and "Blindfold" Scoring

Spice	Evaluation Method	Flavor Scores					
		Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents
Cassia in Pumpkin	Standard known	90	79 ± 0.4	76 ± 2.3	None	65 ± 2.6	None
	Standard unknown	89 ± 2.6	74 ± 4.2	73 ± 3.3	None	68 ± 3.7	None
	Adjusted to basis of 90	90	75	74	None	69	None
Cloves in Pumpkin	Standard known	90	68 ± 1.5	72 ± 2.0	None	63 ± 1.8	None
	Standard unknown	90 ± 0.3	73 ± 4.1	79 ± 3.0	None	68 ± 1.5	None
	Adjusted to basis of 90	90	73	79	None	68	None
Ginger in Pumpkin	Standard known	90	62 ± 6.1	87 ± 3.0	None	74 ± 1.4	68 ± 3.5
	Standard unknown	85 ± 2.3	68 ± 3.6	82 ± 2.9	None	77 ± 2.2	70 ± 3.7
	Adjusted to basis of 90	90	72	87	None	82	74
Nutmeg in Eggnog	Standard known	90	66 ± 5.9	81 ± 4.9	None	71 ± 6.3	None
	Standard unknown	89 ± 0.5	70 ± 3.2	81 ± 2.0	None	68 ± 3.9	None
	Adjusted to basis of 90	90	71	82	None	69	None
Paprika in Cream Sauce	Standard known	90	None	None	74 ± 5.0	None	None
	Standard unknown	85 ± 2.5	None	None	78 ± 2.7	None	None
	Adjusted to basis of 90	90	None	None	83	None	None
Mean of all Scores	Standard known	90	69 ± 3.5	79 ± 3.2	74	68 ± 2.6	68
	Standard unknown	88 ± 1.1	71 ± 1.4	79 ± 2.0	78	70 ± 2.3	70
	Adjusted to basis of 90	90	73	81	83	72	74

TABLE 10

Evaluation of Spices and Spice Substitutes, in Representative Foods, Using Elevated Levels of Spice Substitutes Greater Than Those Recommended By the Manufacturers

Spice	Test Food	Flavor Scores, Using Elevated Levels of Spice Substitutes					
		Natural Spice	Extractives on Sugar or Salt Base	Oils	Oleoresins	Oils with Emulsifying Agents	Oleoresins with Emulsifying Agents
Cassia, increased to	Pumpkin	....	140%	125%	....	175%	....
Scores		90	81 ± 1.3	76 ± 1.7	None	75 ± 2.0	None
Ginger, increased to	Pumpkin	....	160%	100%	....	187%	150%
Scores		90	74 ± 2.9	79 ± 2.2	None	68 ± 3.0	75 ± 1.1
Nutmeg, increased to	Eggnog	....	167%	100%	....	208%	....
Scores		90	73 ± 2.3	84 ± 2.2	None	77 ± 2.2	None
Black Pepper, up to	Cream Sauce	....	147%	167%	111%	117%	150%
Scores		90	75 ± 2.0	78 ± 1.8	77 ± 2.4	81 ± 1.5	74 ± 2.3

Evaluations, using increased levels of spice substitutes greater than manufacturers' recommendations, are given in Table 10. Increasing the amount of extractives as much as feasible to strengths determined to be equal to those of natural spices does not result in scores as high as the scores of the natural spice.

Conclusions

Quantitative evaluation of the strength and quality of the flavor of spices and spice substitutes, either directly or in various test foods, by organoleptic panel methods, is quite feasible, whereas no presently known chemical or physical methods are suitable for this purpose. Sim-

ple statistical analyses of flavor scores obtained by a four-member organoleptic panel on a number of spices and spice substitutes show reasonable reliability and reproducibility of results. General agreement between the results obtained for the same spices and spice substitutes in different test foods confirms the dependability of the method. Reliability and precision of flavor scores obtained by this method tend to increase with added experience of panel members, repeated scoring or use of a larger panel, and refinement of techniques.

The scoring system and the standards of value used in the spice evaluation work here reported were designed to reflect criteria of consumer acceptability. It has been shown in related flavor evaluation work, where we were able to compare the results of panel scoring directly with those of consumer acceptability surveys, that there exists high positive correlation. It is our belief that the panel scores in this paper indicate the relative levels of consumer acceptability of the various spices and spice substitutes examined. Processed foods of similar nature to these test foods, containing these materials, would have consumer acceptance relative to the scores found.

Spice substitutes in general, including oils, oleoresins, sugar- and salt-based extractives, and oils and oleoresins with added emulsifying agents, were found to be of poorer quality in odor and flavor than the corresponding natural spices, whether scored alone or in any of various test foods. The extraction of the essential oils and/or oleoresins from natural spices fails to yield all of the true flavor components. Furthermore, there is evidence of alteration or deterioration of flavor due to sorption bases or added emulsifiers in some spice substitutes, and to oxidation and rancidity in others. It appears reasonable to assume that, in general, separation of the flavor-bearing components of natural spices from their original cellular loci, replete with natural anti-oxidants and other protective factors, results in alteration and reduced stability of the flavor. This is shown most conclusively when comparative odor and flavor evaluations are made directly between natural spices and spice substitutes, both on the straight flavoring materials and in various test foods, under carefully controlled equivalent conditions. Even when unusually large amounts of the spice substitutes—greater than the manufacturers' recommended levels—were used, the spice substitutes failed to "measure up" in quality.

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The Nutritive Value of Canned Foods. III. Changes in Riboflavin Content of Vegetables During Storage Prior to Canning[a]

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Changes in the riboflavin content of either fresh or canned vegetables due to storage prior to canning were small. Vegetables, such as lima beans and corn, that deteriorate readily retained more riboflavin when the storage period was short. There was no loss of riboflavin in canned carrots. Other vegetables retained 41 to 75 percent of the riboflavin found in the fresh samples.

This is the third of a series of papers dealing with the nutritive value of canned foods in which the effect of storage in the periods between harvesting and the canning process, was investigated. The effect of prescribed storage conditions on riboflavin content is reported in this paper.

Little work has been done on the changes in B vitamin content of fresh vegetables during storage before processing. Gleim et al. *(7)* reported higher values for all the vitamins in asparagus and spinach at the end of the growing season. Eheart and others *(2)* found that room storage for as long as 48 hours did not lower significantly the riboflavin content of shelled lima beans.

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[b] The contribution of Dr. Roy E. Marshall and William F. Robertson in supplying the samples is acknowledged.

Riboflavin content of mature beans was 37 to 55 percent less than that of young beans, the amount depending on the location in which the beans were grown. Flynn and others *(6)* and Lamb *(10)* found that the riboflavin in snap beans decreased with increasing maturity. Lamb also reported that there was no significant loss of riboflavin in green beans during holding before processing and that retention was high in corn during all canning operations. Others [Fincke *(5)*, Fellers and others *(4)*, Guerrant and others *(9)*, and Wagner and others *(15)*] reported that the greatest loss of the B vitamins, including riboflavin, was due to the blanching process rather than to a period of holding after the harvest.

Values for percentage retention of riboflavin after canning have varied depending on the kind of vegetable. Fellers, and others *(4)* reported 98 percent retention in canned asparagus, 100 percent in peas, 70 percent in lima beans and 45 percent in canned spinach. Farrell and Fellers *(3)* found 97 percent retention of riboflavin in canned green beans. Guerrant et al. *(8)* reported 78 to 89 percent retention in canned carrots, 39 to 57 percent in peas, 54 to 81 percent in spinach and no loss in canned corn. Wagner et al. *(14)* observed 63 to 103 percent retention in canned asparagus, peas, green beans, lima beans and corn. The range in retention for different samples of each vegetable was wide, due to differences in blanching methods. Lamb et al. *(11)* found little loss in riboflavin content of canned corn and 85 to 106 percent retention in green beans.

Experimental Methods

Details concerning varieties of vegetables used, the methods of growing, harvesting, holding times and sampling procedures have been reported [Marshall and Robertson *(12)*, Paul and others *(13)*].

The field sample of each vegetable was obtained by hand picking at random through the field. It was brought directly to the laboratory where it was cleaned and analyzed immediately. All other samples were harvested by the usual commercial methods. The rest of the samples were stored under defined conditions for different lengths of time. Part of each sample, except the field sample, was canned at the end of the storage period. The times given in the results and in the tables refer to the period between harvesting and processing [Marshall and Robertson *(12)*].

Unprocessed fresh vegetable samples of from five to ten pounds were brought to the laboratory for analysis. After necessary cleaning, the sample was mixed thoroughly and subsampled. Five subsamples of peas, four subsamples of corn and three subsamples of the other vegetables were taken for analysis. One hundred fifty to 200 grams of the vegetable were blended with an equal amount of 0.2N sulphuric acid for three to seven minutes. The samples were transferred to screwtop bottles, toluene added to prevent mold growth and stored in the dark at 0° to 5° centgrade for later analysis. The canned samples were stored for one month, opened, drained and the liquid and solid portions weighed. The solids and liquids were analyzed separately. Riboflavin values in the total can content were calculated on the proportion of the moist weight of solids and liquids in the can. Duplicate samples were blended with 0.2N sulphuric acid, toluene added and stored as above. Total solids were obtained by drying a portion of each sample at 70° centigrade in an oven with circulating air.

At the time of analysis, the sample bottles were inverted slowly 50 times to insure thorough mixing. A weighed amount of the macerated sample was steam hydrolyzed for one hour, then incubated overnight at 45° centigrade with takadiastase and papain enzymes at pH 4.5. Riboflavin was analyzed by the Connor and Straub *(1)* method with "Florisil"[e] (80/100 mesh) as the adsorbent. The degree of flourescence of the sample was measured in a photofluorometer.[f]

Canning losses, as reported, are a total of all losses including those due to blanching.

Results

The riboflavin content of fresh and canned vegetables is reported in Tables 1 to 6. Riboflavin values for the raw vegetables are given as micrograms per gram of fresh weight and of dry weight. Riboflavin values of the

[e] Floridin Company, Warren, Pa.
[f] Lumetron, Will Corporation, Rochester, New York.

TABLE 1

Riboflavin values for green beans (Landreth's Stringless Greenpod)

Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
		Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
	hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Field sample	0	0.12	1.14	10.9	[c]	[c]	[c]
Prompt handling	1.6-2.1	0.12	1.10	10.4	0.08	0.89	8.5
Shade	5-6	0.11	0.89	11.9	[d]	[d]	[d]
	11	0.11	0.91	12.0	[d]	[d]	[d]
	23-24	0.13	1.14	11.6	0.08	0.98	8.5
	48	0.13	1.12	11.6	0.08	0.86	8.8
Cold storage	11	0.12	1.20	10.3	0.08	0.75	10.7
	23-24	0.13	1.16	11.1	0.08	0.93	8.8
	48	0.13	1.12	10.8	0.07	0.76	9.6
	72	0.12	1.03	11.9	[d]	[d]	[d]
	120	0.13	1.21	10.4	0.08	0.92	9.2
Snow ice	48	0.13	1.23	10.7	0.08	0.91	8.5
	72	0.13	1.00	12.8	0.08	0.89	9.3
	120	0.11	1.10	10.2	0.08	0.87	8.7

[c] Not canned. [d] Not determined.

total can contents are reported as micrograms per gram on both the moist weight and the dry weight basis.

Green Beans. Two pickings of green beans (Landreth's Stringless Greenpod) were analyzed (Table 1).

The change in riboflavin content of fresh green beans during the storage periods before canning was small. There was some loss in riboflavin in the samples held five to 11 hours in outdoor shade followed by a slight increase at the end of 24 hours in the shade. The changes did not appear to be related to loss of moisture. The green beans held in snow ice for 120 hours were slightly lower in riboflavin than the field sample.

The kinds of pre-canning treatments used had no effect on the riboflavin content of the canned beans and there was a retention of 58 to 68 percent of the riboflavin in the total can contents after the canning process. The variation between storage samples was greater when canned green bean values were calculated on the dry weight basis but this variation in riboflavin content did not show any specific trend.

Lima Beans. Two lots of lima beans, Thorogreen variety, were harvested. The second lot was picked three days after the first lot in a different portion of the same field. Riboflavin values for the two lots are averaged in Table 2 since the data for the two series did not differ.

The field sample of lima beans was higher in riboflavin than the samples handled promptly or than any of the stored samples. Although the samples held in outdoor shade showed little variation in riboflavin content when expressed on the moist weight basis, the samples lost moisture during holding and consequently were lower in riboflavin when expressed on a dry weight basis than the samples handled promptly. Samples washed and then stored in the shade lost a greater amount of riboflavin. The lima beans which had been dipped in calcium propionate solution before storage in the shade retained approximately the original amount of riboflavin. The sample held for 24 hours in cold storage was slightly lower in riboflavin but the riboflavin increased during the longer periods of cold storage. All of these changes were too small to be of much importance in determining the best storage method. The sample held in running water for 24 hours was lower in riboflavin than any other sample.

The riboflavin content of the canned lima beans depended to some extent on the type of storage prior to canning. The lowest riboflavin value was found in the samples which had been held in running water or which had been washed and held in the shade before they were canned. The average retention of riboflavin in the total can contents during the canning process was 45 percent. This is lower than the 63 to 97 percent retention found in canned lima beans by Wagner et al. *(14)*. The reason for this discrepancy could not be determined.

Carrots. One lot of Red Cored Chantenay carrots was harvested and divided into three parts. The first sample was analyzed immediately, the remainder was canned. The other two samples were stored, one in cold storage, the other in a pile outdoors covered with straw. Samples were taken from storage at the end of one week, one month and three months [Marshall and Robertson *(12)*]. Riboflavin analyses were made on the sample handled promptly, one sample stored for one week outdoors, one sample stored three months outdoors and one sample stored three months in cold storage. The riboflavin content of raw and canned carrots is given in Table 3.

Fresh carrots showed a gain in riboflavin after three months cold storage which was not due to a change in moisture content. The lowest riboflavin content was in the sample stored three months in the pit. The same relationship was found in the canned samples. When the riboflavin content of the sample canned after three months cold storage was compared to the fresh carrots which had been handled promptly, the canned sample

TABLE 2

Riboflavin values for lima beans (Thorogreen)

Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
		Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
	hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Field sample	0	0.17	0.48	34.6	[a]	[a]	[a]
Prompt handling	1.5-1.9	0.15	0.43	35.5	0.05	0.16	29.3
Shade	5.2	0.15	0.40	37.9	0.05	0.18	26.8
	8.2-9.2	0.15	0.36	41.8	[b]	[b]	[b]
	24	0.15	0.37	39.7	0.05	0.17	28.1
Running water	8.2-9.2	0.14	0.40	33.5	0.05	0.17	27.3
	24	0.12	0.31	38.7	0.04	0.16	26.8
Washed, shade	8.2-9.2	0.13	0.33	38.9	[b]	[b]	[b]
	24	0.13	0.38	34.4	0.04	0.14	27.1
10% CaPr,[1] shade	24	0.15	0.43	34.8	0.05	0.18	27.7
Cold storage	24	0.13	0.33	39.0	0.05	0.18	28.1
	48	0.15	0.37	40.4	[a]	[a]	[a]
	120	0.15	0.39	38.6	[a]	[a]	[a]
Washed, cold storage	120	0.14	0.38	36.4	[a]	[a]	[a]
10% CaPr,[1] cold storage	120	0.15	0.37	39.0	[a]	[a]	[a]
Snow ice	24	0.15	0.39	38.4	0.05	0.17	28.4
	120	0.17	0.39	42.0	[a]	[a]	[a]

[a] Not canned. [b] Not determined. [1] Calcium propionate.

TABLE 3

Riboflavin content of carrots
(Red Cored Chantenay)

Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
		Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
	days	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Prompt handling	0	0.05	0.40	11.4	[1]	[1]	[1]
Outdoors, straw covered	7	0.06	0.52	10.5	0.04	0.44	8.6
	90	0.04	0.30	11.8	0.04	0.41	8.5
Cold storage	90	0.06	0.61	9.7	0.05	0.63	8.6

[1] Not canned.

TABLE 4

Riboflavin values for corn

Variety	Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
			Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
		hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Seneca Golden	Field sample	0	0.09	[l]	[l]	[k]	[k]	[k]
	Prompt handling	2.3	0.09	0.27	33.2	0.04	[l]	[l]
	Held on wagon	5.9	0.08	0.25	31.3	0.04	[l]	[l]
		8.7	0.08	0.25	31.8	0.05	[l]	[l]
		25.5	0.08	0.25	33.0	0.04	[l]	[l]
Golden Cross Bantam	Field sample	0	0.09	0.31	28.1	[k]	[k]	[k]
	Prompt handling	2.0	0.09	0.36	25.3	0.07	0.48	15.0
	Held on wagon	4.9	0.09	0.34	26.2	0.07	0.43	15.5
		7.4	0.08	0.32	26.0	0.05	0.32	15.3
		23.8	0.09	0.33	27.3	0.05	0.31	16.5

[k] Not canned. [l] Not determined.

TABLE 5

Riboflavin values for peas

Variety	Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
			Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
		hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Thomas Laxton	Field sample	0	0.12	0.57	20.2	[m]	[m]	[m]
	Prompt handling	2.3	0.12	0.62	19.8	0.08	0.36	14.7
	Shade	5.5	0.12	0.78	15.0	0.08	0.41	12.9
	Washed, shade	5.5	0.12	0.62	18.4	[n]	[n]	[n]
	Unvined	4.3	0.14	0.81	17.8	[m]	[m]	[m]
	Running water	9.3	0.14	0.92	15.3	0.08	0.37	13.9
	Cold storage	9.4	0.14	0.76	18.8	0.08	0.39	14.5
		22.8	0.16	0.78	20.2	0.08	0.39	14.7
		46.8	0.14	0.73	19.8	0.08	0.36	13.9
	Washed, cold storage	9.4	0.14	0.83	16.3	0.08	0.37	14.3
		22.8	0.13	0.75	17.5	0.08	0.40	12.6
		46.8	0.14	0.76	18.9	[n]	[n]	[n]
	Snow ice	22.8	0.12	0.70	17.8	0.08	0.39	13.2
		46.8	0.12	0.67	18.4	0.07	0.29	12.9
Pride	Field sample	0	0.12	0.60	20.2	[m]	[m]	[m]
	Prompt handling	1.3	0.12	0.60	19.1	0.08	0.33	16.2
	Shade	4.8	0.13	0.61	21.4	0.09	0.32	17.0
		10.3	0.13	0.68	19.6	0.09	0.34	15.7
	Washed, shade	4.8	0.12	0.59	20.8	[n]	[n]	[n]
		10.3	0.14	0.78	18.1	0.09	0.36	14.9
	Running water	10.3	0.12	0.75	16.2	0.08	0.35	14.6
	Cold storage	10.3	0.15	0.76	19.0	0.08	0.32	15.0
		26.0	0.14	0.64	21.2	0.08	0.34	14.3
		48.3	0.14	0.70	20.5	[n]	[n]	[n]
	Washed, cold storage	10.3	0.15	0.72	20.1	0.08	0.30	13.6
		26.0	0.13	0.69	19.2	0.08	0.36	15.1
		48.3	0.15	0.74	19.7	[n]	[n]	[n]
	Snow ice	26.0	0.15	0.76	19.7	0.08	0.33	14.3
		48.3	0.14	0.72	19.8	[n]	[n]	[n]
Shasta	Field sample	0	0.16	0.82	19.4	[m]	[m]	[m]
	Prompt handling	1.2	0.15	0.74	20.2	0.09	0.32	18.8
	Shade	4.4	0.13	0.59	21.2	0.09	0.45	14.5
		7.9	0.14	0.64	21.2	0.08	0.34	15.5
	Washed, shade	4.4	0.11	0.53	20.8	[n]	[n]	[n]
		7.9	0.12	0.66	17.6	[n]	[n]	[n]
	Unvined	17.3	0.13	0.63	21.2	[n]	[n]	[n]
	Running water	7.9	0.13	0.74	17.1	0.08	0.39	14.7
	Cold storage	7.9	0.15	0.77	19.1	0.08	0.38	15.1
		24.2	0.16	0.76	21.1	0.09	0.40	14.9
	Washed, cold storage	7.9	0.14	0.76	17.8	0.10	0.40	15.4
		24.2	0.13	0.71	18.4	0.09	0.37	15.5
	Snow ice	24.2	0.14	0.72	19.5	0.08	0.35	15.3

[m] Not canned. [n] Not determined.

was 11 percent higher in riboflavin than the fresh. The samples stored one week outdoors retained 80 percent of the riboflavin during canning but the samples held for 90 days outdoors increased 35 percent during the canning process.

Corn. The riboflavin content of the two varieties of corn (Seneca Golden and Golden Cross Bantam) which were analyzed are shown in Table 4. The fresh corn of both varieties were similar in riboflavin content. Holding the corn on the wagon for as long as 24 hours before processing did not change the riboflavin values.

All the canned samples of both varieties of corn were 40 to 50 percent lower in riboflavin than the fresh samples except two samples of Golden Cross Bantam corn. These two samples of corn (prompt handling and the sample held on the wagon for 4.9 hours) retained 75 to 80 percent of the riboflavin during the canning operation when comparisons were made on the fresh weight basis. There was no loss in riboflavin when the fresh and canned corn were compared on the dry weight basis.

Peas. The effect of the type of storage before canning on vitamin content was studied in three varieties of peas, Thomas Laxton, Pride and Shasta (Table 5).

The field sample and the sample handled promptly were higher in mean riboflavin in the Shasta peas than in the similar samples of other varieties but the range in riboflavin content was the same for all three varieties. High riboflavin retentions in fresh Thomas Laxton and Shasta peas were found in the samples held in cold storage for 23 to 24 hours. In Pride peas the highest riboflavin content was found in the sample stored 26 hours in snow ice. Other samples which were high in riboflavin were those held in cold storage and those samples that were handled promptly.

The type of storage which was used before canning had little effect on the riboflavin content of the canned peas. There was a 41 to 75 percent retention of riboflavin in the total can contents during the canning process. This retention was a little higher than that reported by Guerrant et al. *(8)*. Though little difference was found in the riboflavin content of canned peas, the shorter periods of storage gave a more acceptable canned product (unpublished data, this laboratory).

Spinach. Two varieties of spinach, one grown on upland soil, one grown on muck soil, were analyzed. The effect of storage of the fresh vegetable on the riboflavin content is given in Table 6. No canned samples were analyzed.

The differences in riboflavin content between the two varieties of spinach were small. All samples of the muck grown spinach increased in riboflavin during storage except the sample held for 72 hours in cold storage. This increase was related to an increase in percentage solids. The riboflavin content of the spinach grown on upland soil remained about the same for all samples except the sample held overnight in the field. This sample was a little higher in riboflavin than the other samples grown on upland soil.

The relatively small change in the riboflavin content of spinach can not be used as a measure of the best type of storage of the fresh vegetable before canning. The first lot of spinach grown on upland soil was grown about 70 miles from the cannery and was harvested the evening before it was sent to the cannery. There was no field sample from this lot and the storage times began 17 hours after the harvesting [Marshall and Robertson *(12)*]. All samples were somewhat deteriorated upon arrival in the laboratory. The muck spinach was grown on the campus farms. The field sample, the sample which was handled promptly and the samples stored for the shortest time periods were fairly fresh but all samples deteriorated rapidly during all types of storage.

Discussion

The conditions under which raw vegetables are stored between harvesting and canning appear to be minor factors influencing the riboflavin content of the canned product. Type of soil, seasonal variations of rainfall and temperature may have some effect on vitamin content. The 1946 growing season was very dry. Some of the vegetables, especially the lima beans, were slow in reaching maturity due to this extreme dryness. Even though care was taken to harvest each lot of the separate vegetables at the same stage of maturity, Marshall and Robertson *(12)* reported variations in this. For instance, Thomas Laxton peas were "satisfactory but

TABLE 6

Riboflavin values for fresh spinach

Method of Holding	Time of Holding After Harvest	Upland			Muck		
		Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
	hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Field sample	0				0.15	2.24	6.5
Prompt handling	2				0.15	1.93	7.6
Overnight in field	17	0.19	2.46	7.6			
Shade, baskets	7				0.20	3.00	6.8
	21	0.17	2.11	8.0			
	24				0.18	2.56	7.0
	37	0.17	2.41	6.9			
	72				0.19	2.66	7.2
Shade, pile	24				0.20	3.01	6.8
	37	0.16	2.12	7.7			
Cold storage	24				0.21	3.04	6.8
	37	0.16	1.95	8.1			
	61	0.17	2.16	7.7			
	72				0.13	2.24	5.9
	133	0.16	2.26	7.2			

could have been left one day," while Pride peas were "one or two days too mature." Though these differences did not affect the riboflavin content in these particular varieties, this example demonstrates the need in the canning industry for more objective methods for measuring maturity in all vegetables.

While there were no storage methods which promoted consistently high retentions of riboflavin in all vegetables, certain conclusions may be drawn. Vegetables that deteriorate readily in eating quality, as corn, peas and spinach tend to have a higher riboflavin content when they are canned soon after harvesting. On the other hand, carrots increased in vitamin content in cold storage but decreased in riboflavin content during pit storage. These differences are not related to a moisture change. The only vegetable in which the riboflavin content and the percentage solids were correlated was the muck grown spinach. In some cases vegetables washed before storage or held in running water were low in riboflavin.

In general, the retention of riboflavin in vegetables was not affected by the type of storage used prior to canning. The greatest change in riboflavin content occurred during the canning process rather than during the storage period.

Summary

The riboflavin content of fresh and canned green beans, lima beans, carrots, corn, peas and of fresh spinach has been investigated. Certain methods of storage prior to canning were studied for their effect on riboflavin retention before and after canning.

Differences in riboflavin content due to type of storage were inconsistent. There was some tendency toward a higher retention in the samples canned soon after the harvest, especially in samples of corn, peas and spinach. Variety differences were not significant in any vegetable.

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The Nutritive Value of Canned Foods. IV. Changes in Thiamine Content of Vegetables During Storage Prior to Canning[a]

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Shorter storage periods prior to canning resulted in higher thiamine retention in both fresh and canned vegetables. Lower thiamine retention was found when vegetables were washed before storage or held in running water. Several vegetables increased in thiamine content during long periods of cold storage without a corresponding increase in percentage solids. The retention of thiamine after the canning process varied with the variety of vegetables.

The effect of various methods of storage prior to canning on the ascorbic acid, carotene, riboflavin and thiamine content of fresh and canned vegetables has been investigated. This paper reports the thiamine data obtained in the study.

Previous studies on the storage of fresh vegetables have shown that little change occurs in thiamine content in the storage period before the vegetable is canned. Moyer and Tressler *(14)* found that there was no loss of thiamine in peas held for 16 hours on the vine in the viner shed plus subsequent holding for three hours between shelling and washing. Eheart et al. *(3)* reported that there was no significant change in the thiamine content of lima beans after holding for 48 hours at room temperature. Lamb *(11)* observed no significant loss of thiamine in green beans due to a holding period between harvesting and canning but did not state the methods of holding.

Gleim and others *(6)* found an increase in the vitamin content of spinach and asparagus at the end of the growing season. Ives et al. *(10)* observed a similar increase in peas. Clifcorn and Heberlein *(2)* reported an increase in the thiamine content of mature lima beans and peas. Eheart and others *(3)* found that an increase in the maturity of lima beans resulted in a decrease in thiamine. Flynn et al. *(5)* reported a continual decrease in the pods of green beans as they matured. Thiamine concentration increased in the seed of the green beans as they matured due to the increase in the percentage solids.

Fellers et al. *(4)*, Lamb et al. *(12)* and Wagner et al. *(16)* reported that the greatest loss in thiamine was due to the blanching process. Bedford and McGregor *(1)* reported similar findings. Increasing the time of the blanch increased the loss of the vitamin. This was thought by these authors to be due to the leaching of the thiamine from the vegetable.

The retention of thiamine after canning depended on the kind of vegetable. Guerrant and coworkers *(7)* found the lowest retention of thiamine in corn and spinach after canning in which case only 23 to 34 percent of the vitamin was retained. Green beans, lima beans and carrots each retained about 70 percent of the vitamin and peas lost little thiamine during the canning process. Wagner, Strong and Elvehjem *(16)* reported a similar percentage retention in corn and green beans but found 52 to 70 percent retention in canned peas and 32 to 60 percent retention of thiamine in canned lima beans.

Experimental Procedure

Details concerning the growing, harvesting, sampling and storage of vegetables included in this paper were reported by Marshall and Robertson *(13)* and by Paul et al. *(15)*.

The preparation of the vegetable samples (subsampling, acid hydrolysis and enzymatic digestion)

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TABLE 1

Thiamine values for green beans (Landreth's Stringless Greenpod)

Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
		Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
	hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Field sample	0	0.10	0.95	10.9	[e]	[e]	[e]
Prompt handling	1.6-2.1	0.10	0.92	10.4	0.05	0.57	8.5
Shade	5-6	0.11	0.88	11.9	[f]	[f]	[f]
	11	0.11	0.94	12.0	[f]	[f]	[f]
	23-24	0.10	0.89	11.6	0.07	0.81	8.5
	48	0.11	0.92	11.6	0.08	0.89	8.8
Cold storage	11	0.12	1.12	10.3	0.07	0.64	10.7
	23-24	0.11	0.96	11.1	0.05	0.56	8.8
	48	0.11	0.97	10.8	0.05	0.53	9.6
	72	0.11	0.92	11.9	[f]	[f]	[f]
	120	0.15	1.46	10.4	0.04	0.44	9.2
Snow ice	48	0.10	0.94	10.7	0.04	0.51	8.5
	72	0.11	0.85	12.8	0.06	0.63	9.3
	120	0.09	0.84	10.2	0.06	0.63	8.7

[e] Not canned. [f] Not determined.

for thiamine analysis was the same as for riboflavin analysis (Ingalls et al. *(9)*. Thiamine was determined by the thiochrome method described by Hennessy *(8)*. Short adsorption columns were used with "Decalso" [c] (60/80 mesh) as the adsorbent. After conversion of the thiamine to thiochrome, the degree of fluorescence produced by the thiochrome was measured in a photofluorometer.[d] Sample blanks were made by omission of the conversion of thiamine to thiochrome.

Results

Thiamine values for freshly harvested, stored and canned green beans, lima beans, carrots, corn, peas and for fresh spinach are reported in Tables 1 to 6. The thiamine content of unprocessed vegetables is reported as micrograms per gram on both the raw and dry weight basis. The thiamine values of the total can content are reported as micrograms per gram on both the moist and the dry weight basis.

Green Beans. Two lots of green beans (Landreth's Stringless Greenpod) were picked in the same field five days apart. Since there was no difference in the thiamine values, the averages of the two pickings, fresh and canned, are reported in Table 1.

The differences in thiamine content were small among the raw samples stored under the various defined conditions. The highest thiamine value was obtained in the sample held for 120 hours in cold storage while the raw green beans held for 120 hours in snow ice were the lowest in thiamine. There was no corresponding change in percentage moisture.

Canned green beans were 26 to 57 percent lower in thiamine than unprocessed green beans except for the sample stored 120 hours in cold storage before canning. This sample was 74 percent lower in thiamine than the raw green beans. Two samples canned 24 and 48 hours after shade holding retained the greatest amount of the vitamin. When comparison of the raw and canned beans of these two samples was made on the dry weight basis, there was little change in thiamine after canning. Generally, the effect of variations in holding before canning on the thiamine content of the canned product were small as compared with the loss of thiamine during the canning procedure.

Lima Beans. The thiamine content of raw and canned lima beans (Thorogreen) is reported in Table 2.

Certain methods of storage after the harvest resulted in low thiamine values in the raw lima beans. The main difference was between the samples which had been washed and the samples which had not been washed before the storage period. Samples washed and held in the shade, washed before cold storage, held in running

[c] Permutite Co., N. Y.
[d] Lumetron, Will Corp., Rochester, N. Y.

TABLE 2

Thiamine values for lima beans (Thorogreen)

Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
		Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
	hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Field sample	0	0.39	1.13	34.6	[g]	[g]	[g]
Prompt handling	1.5-1.9	0.32	0.90	35.5	0.06	0.20	29.3
Shade	5.2	0.34	0.91	37.9	0.06	0.21	26.8
	8.2-9.2	0.28	0.67	41.8	[h]	[h]	[h]
	24	0.33	0.84	39.7	0.03	0.09	28.1
Running water	8.2-9.2	0.28	0.83	33.5	0.02	0.07	27.3
	24	0.24	0.62	38.7	0.01	0.05	26.8
Washed, shade	8.2-9.2	0.31	0.80	38.9	[h]	[h]	[h]
	24	0.29	0.83	34.4	0.02	0.06	27.1
10% CaPr,[i] shade	24	0.30	0.87	34.8	0.02	0.08	27.7
Cold storae	24	0.37	0.95	39.0	0.05	0.16	28.1
	48	0.30	0.75	40.4	[g]	[g]	[g]
	120	0.33	0.84	38.6	[g]	[g]	[g]
Washed, cold storage	120	0.20	0.55	36.4	[g]	[g]	[g]
10% CaPr,[i] cold storage	120	0.20	0.50	39.0	[g]	[g]	[g]
Snow ice	24	0.30	0.78	38.4	0.02	0.09	28.4
	120	0.23	0.55	42.0	[g]	[g]	[g]

[g] Not canned. [h] Not determined. [i] Calcium propionate.

TABLE 3

Thiamine content of carrots (Red Cored Chantenay)

Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
		Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
	days	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Prompt handling	0	0.05	0.41	11.4	[j]	[j]	[j]
Outdoors, straw covered	7	0.05	0.49	10.5	0.04	0.42	8.6
	90	0.02	0.15	11.8	0.02	0.22	8.5
Cold storage	90	0.02	0.23	9.7	0.03	0.34	8.6

[j] Not canned.

water and samples held for the longer period in snow ice were low in thiamine. The samples which had been dipped in calcium propionate before cold storage retained the least amount of the vitamin. Changes in the moisture content of the lima beans were not related to these changes in the thiamine content.

The retention of thiamine in the lima beans during the canning process was 5 to 22 percent. This is considerably lower than Guerrant et al. *(7)* reported. The effect of the type of storage of the vegetable prior to canning was apparent in the canned lima beans. Samples handled promptly or held for short periods in the shade and in cold storage were higher in thiamine than the other samples. However, the differences in thiamine in the bean samples canned after the various storage methods were small compared to the amount of thiamine remaining after the canning process.

Carrots. Four samples of raw and canned carrots, Red Cored Chantenay, were analyzed for thiamine (Table 3). One sample was analyzed immediately after the harvest. Two samples were held seven and 90 days in a straw covered pile outdoors and the fourth sample was held in cold storage for 90 days.

The effect of the long storage of carrots on the thiamine content was evident in both the raw and the canned vegetable. Storage in the pile for one week did not affect the thiamine in the carrot, since this sample and the sample handled promptly were approximately

TABLE 4

Thiamine values for corn

Variety	Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
			Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
		hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Seneca	Field sample	0	0.15	[l]	[l]	[k]	[k]	[k]
	Prompt handling	2.3	0.16	0.50	33.2	0.03	[l]	[l]
	Held on wagon	5.9	0.14	0.45	31.3	0.04	[l]	[l]
		8.7	0.17	0.53	31.8	0.04	[l]	[l]
		25.5	0.18	0.56	33.0	0.04	[l]	[l]
Golden Cross Bantam	Field sample	0	0.16	0.58	28.1	[k]	[k]	[k]
	Prompt handling	2.0	0.15	0.60	25.3	0.03	0.23	15.0
	Held on wagon	4.9	0.16	0.60	26.2	0.02	0.16	15.5
		7.4	0.13	0.51	26.0	0.03	0.16	15.3
		23.8	0.17	0.61	27.3	0.02	0.12	16.5

[k] Not canned. [l] Not determined.

TABLE 5

Thiamine values for peas

Variety	Method of Holding	Time of Holding After Harvest	Before Processing			Total Can Contents		
			Raw Weight	Dry Weight	Percent Solids	Moist Weight	Dry Weight	Percent Solids
		hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Thomas Laxton	Prompt handling	2.3	0.32	1.62	19.8	0.19	1.51	14.7
	Shade	5.5	0.33	2.17	15.0	0.18	1.51	12.9
	Washed, shade	5.5	0.34	1.87	18.4	[n]	[n]	[n]
	Unvined	4.3	0.36	2.14	17.8	[n]	[n]	[n]
	Rnning water	9.3	0.28	1.81	15.3	0.16	1.32	13.9
	Cold storage	9.4	0.35	1.87	18.8	0.21	1.62	14.5
		22.8	0.37	1.82	20.2	0.19	1.51	14.7
		46.8	0.38	1.92	19.8	0.18	1.56	13.9
	Washed, cold storage	9.4	0.34	2.10	16.3	0.18	1.43	14.3
		22.8	0.38	2.18	17.5	0.22	1.90	12.6
		46.8	0.36	1.90	18.9	[n]	[n]	[n]
	Snow ice	22.8	0.36	2.01	17.8	0.15	1.21	13.2
		46.8	0.40	2.16	18.4	0.18	1.60	12.9
								[m]
Pride	Field sample	0	0.48	2.38	20.2	[m]	[m]	16.2
	Prompt handling	1.3	0.49	2.56	19.1	0.19	1.39	17.0
	Shade	4.8	0.51	2.37	21.4	0.20	1.33	15.7
		10.3	0.52	2.63	19.6	0.18	1.39	[n]
	Washed, shade	4.8	0.48	2.31	20.8	[n]	[n]	14.9
		10.3	0.45	2.47	18.1	0.25	1.93	14.6
	Rnning water	10.3	0.43	2.65	16.2	0.18	1.56	15.0
	Cold storage	10.3	0.49	2.60	19.0	0.22	1.64	14.3
		26.0	0.48	2.28	21.2	0.15	1.36	[n]
		48.3	0.52	2.53	20.5	[n]	[n]	13.6
	Washed, cold storage	10.3	0.44	2.18	20.1	0.16	1.42	15.1
		26.0	0.42	2.17	19.2	0.15	1.31	[n]
		48.3	0.47	2.38	19.7	[n]	[n]	14.3
	Snow ice	26.0	0.46	2.34	19.7	0.20	1.67	[n]
		48.3	0.49	2.46	19.8	[n]	[n]	
								[m]
Shasta	Field sample	0	0.36	1.86	19.4	[m]	[m]	18.8
	Prompt handling	1.2	0.45	2.24	20.2	0.20	1.12	14.5
	Shade	4.4	0.48	2.26	21.2	0.18	1.35	15.5
		7.9	0.50	2.34	21.2	0.17	1.28	[n]
	Washed, shade	4.4	0.34	1.65	20.8	[n]	[n]	[n]
		7.9	0.44	2.51	17.6	[n]	[n]	[n]
	Unvined	17.3	0.58	2.74	21.2	[n]	[n]	14.7
	Rnning water	7.9	0.40	2.34	17.1	0.18	1.43	15.1
	Cold storage	7.9	0.40	2.10	19.1	0.20	1.51	14.9
		24.2	0.44	2.07	21.1	0.22	1.65	15.4
	Washed, cold storage	7.9	0.38	2.11	17.8	0.17	1.36	15.5
		24.2	0.48	2.60	18.4	0.19	1.44	15.3
	Snow ice	24.2	0.49	2.49	19.5	0.17	1.33	

[m] Not canned. [n] Not determined.

the same in thiamine content. When the carrots were held in cold storage for 90 days about half of the thiamine was retained. The sample held 90 days in the straw pile retained about 38 percent of the vitamin. There was an apparent increase in thiamine content during the canning of this sample when the raw and canned carrots were compared on the dry weight basis. The thiamine content of canned carrots was about the same as the comparable raw sample. Losses due to the canning operation were negligible.

Corn. The thiamine content of two varieties of corn, Seneca Golden and Golden Cross Bantam, is reported in Table 4.

Though the amount of thiamine in the two varieties of corn were about the same, some differences appeared in the corn held before canning. The field sample and the sample marked "prompt handling" were similar in thiamine content. The samples held on the wagon for 24 to 26 hours increased slightly in thiamine content above the original amount. The sample of Golden Cross Bantam corn held for seven hours on the wagon retained a little less than the original amount.

All canned samples of Seneca Golden corn contained comparable amounts of the thiamine. The retention of thiamine in these samples during the canning process was 20 to 25 percent. Golden Cross Bantam corn retained 10 to 23 percent of the thiamine. In the three samples of Golden Cross Bantam corn which had been held on the wagon before they were canned, the amount of thiamine in the canned product decreased with an increasing length of storage time.

Peas. The thiamine content of three varieties of fresh and canned peas, Thomas Laxton, Pride and Shasta, is given in Table 5.

Thomas Laxton peas were lower in thiamine than the other varieties but there were no differences in the thiamine content of Pride and Shasta peas. The effect of the different pre-canning storage methods and times on the thiamine content of the raw peas was not consistent for all varieties. Certain trends in thiamine retention after the various storage methods were noted, however. A sample of Thomas Laxton peas and one of Shasta peas were left on the vines in the field after the mowing. Though the length of time the Shasta peas were held on the vines was longer than for the Thomas Laxton peas, both samples were high in thiamine. The unvined Shasta peas increased in thiamine content during the holding period.

The lowest thiamine retentions were found in the peas held eight to ten hours in running water or in those samples which were washed before the holding periods. Thiamine values had a tendency to increase during storage. This increase was apparent in all types of storage and was related in most cases to a corresponding increase in the percentage solids.

There was 50 to 80 percent retention of thiamine after the canning process except for one sample which retained 93 percent of the thiamine. This last sample was the sample of Thomas Laxton peas which had been handled promptly. As was true with the other vegetables, the loss due to the type of storage before the canning process was insignificant when the losses due to the canning operations were considered. Highest values of thiamine were found in the samples which were canned after cold storage or after prompt handling. Lower thiamine values were observed in the samples canned after holding in running water or after washing previous to cold storage. There are some exceptions. For example, the sample of canned Pride peas which was washed before shade storage was higher in thiamine than the cold storage samples of the same variety.

Spinach. Two varieties of spinach were studied. One was a winter variety grown on upland soil. The other variety was from a spring planting on muck soil. The effects of several methods of storage on the vitamin content of the fresh spinach are shown in Table 6. No canned samples were analyzed.

The thiamine content of the two varieties of spinach was about the same. Direct comparisons were impossible since the first sample of upland spinach was received 17 hours after the harvest and after it had been shipped about 70 miles. The muck grown spinach was from the campus farms and was available for analysis directly from the fields [Ingalls et al. *(9)*]. The samples of upland spinach which had been held in the shade in baskets had little thiamine remaining at the end of 37 hours. The thiamine content of this variety was high at the end of 133 hours in cold storage.

TABLE 6

Thiamine values for raw spinach

Method of Holding	Time of Holding After Harvest	Upland			Muck		
		Raw Weight	Dry Weight	Percent Solids	Raw Weight	Dry Weight	Percent Solids
	hrs.	*mg./100 gm.*	*mg./100 gm.*		*mg./100 gm.*	*mg./100 gm.*	
Field sample	0				0.05	0.83	6.5
Prompt handling	2				0.05	0.63	7.6
Overnight in field	17	0.08	1.03	7.6			
Shade, baskets	7				0.07	1.03	6.8
	21	0.07	0.91	8.0			
	24				0.04	0.56	7.0
	37	0.01	0.19	6.9			
	48				0.04	0.58	7.2
Shade, pile	24				0.08	1.12	6.8
	37	0.02	0.26	7.7			
Cold storage	24				0.03	0.38	6.8
	37	0.06	0.74	8.1			
	61	0.06	0.73	7.7			
	72				0.04	0.66	5.9
	133	0.08	1.14	7.2			

The muck grown spinach, on the other hand, was highest in thiamine content after 24 hours in a pile in the shade.

These findings have little importance since the eating quality of the spinach is destroyed by long storage of the raw vegetable. Spoilage was extensive in all but the samples held for the shortest time periods. It is impractical to ignore this deterioration in any discussion of the use of this vegetable as a food.

Discussion

Certain factors appear to affect the retention of thiamine in raw and canned vegetables. The type of storage employed after the harvest, the maturity of the vegetable, the rainfall during the growing season and condition of the soil may determine to some extent the vitamin content of the vegetable. The handling of the vegetable after the harvest seems to be one of the more important factors. Though the findings of the present study do not point out certain holding methods which excel for each vegetable, some indication of which of the holding procedures were more acceptable was found. Washing of the vegetable before pre-canning storage or holding in running water resulted in thiamine losses in most kinds of vegetables. Presumably some thiamine was dissolved during the washing. Wagner et al. *(16)* observed this leaching of the vitamin out of the vegetable during the blanching process. Most of the vegetables increased in thiamine during long periods in cold storage without a corresponding increase in percentage solids. Carrots held for 90 days in cold storage were an exception since this sample retained only 50 percent of the thiamine. The variety of vegetable was not a factor except in the case of peas. Several authors [Clifcorn and Heberlien, *(2)*; Ives et al. *(10)*] observed an increase in thiamine in peas with increasing maturity. The Thomas Laxton peas used in this study were slightly immature [Marshall and Robertson *(13)*] and were also lower in thiamine than the other varieties. The effect of growing conditions on the vitamin content could not be determined since the vegetables used in this study were harvested during one growing season.

The percentage retention of thiamine during the canning operation (including the usual cutting, washing and blanching) was low for green beans, lima beans, peas and corn. Some samples of canned lima beans and canned corn retained as little as five to 15 percent of the vitamin. Carrots were the only vegetable with the same amount of vitamin in the raw and canned samples. In general, differences in the thiamine content of the samples of vegetables resulting from the various storage conditions were small when compared to canning losses. Since the samples canned after prompt handling or after short storage periods were the best canned product from the standpoint of appearance and flavor (unpublished data, this laboratory), these factors must be included in the consideration of the best pre-canning storage procedures.

Summary

The thiamine content of green beans, lima beans, carrots, corn, peas and spinach has been reported. The effect of various methods of storage between the harvest and processing on the thiamine content of the vegetables was included.

No specific storage method or time was found to be the best for all vegetables. In general, longer storage periods resulted in an increase of thiamine in the raw and canned vegetables. Washing before storage or holding in running water resulted in a decrease in thiamine in some vegetables. The retention in thiamine during the canning operation was less than the retention during pre-canning storage except in the case of canned carrots.

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The Nutritive Value of Canned Foods. V. Changes in Carotene Content of Vegetables During Storage Prior to Canning[a]

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Carotene content of carrots, peas and spinach was determined at harvest, under various conditions of holding prior to canning and after canning. Retention of carotene during precanning holding and after canning was high in all cases.

Previous papers in this series deal with handling procedures prior to commercial canning of vegetables and changes occurring in ascorbic acid, riboflavin and thiamine content of vegetables subjected to accepted commercial canning procedures. This paper reports results of studies on changes in the carotene content during handling procedures prior to canning of those vegetables included in the previous reports which were considered fair to good sources of carotene. The vegetables analyzed for carotene were carrots, peas and spinach.

Gleim, Tressler and Fenton *(1)* reported little loss of carotene in spinach during 24 hour storage at 18.8-25.5° C. and no appreciable carotene loss in spinach after a week's storage at 0-4.4° C. Stimson, Tressler and Maynard *(10)* reported that vining, blanching and other processes incidental to preparation for freezing caused the loss of little or no carotene content from peas. Lamb, Pressley and Zuch *(3)* reported apparent increases in the carotene content of both peas and carrots during canning operations. Other studies by McKillican *(6)* and Lee and Tapley *(4)* on effects of root cellar storage on carotene in carrots showed good retention of the carotene during root cellar storage. Guerrant and others *(2)* found that during commercial canning, carotene retention was excellent in spinach and carrots and good in peas.

Product Handling and Analytical Methods

Details of growing, harvesting, handling and laboratory sampling are given by Marshall and Robertson *(5)* and Paul and others *(7)* in the first two papers of this series.

One lot of Red Cored Chantenay, muck grown carrots was harvested. Two kilogram samples of each subsample of carrots were brought to the laboratory. These were put through a commercial "gricer" and the grated carrot sample was thoroughly mixed for subsampling for carotene, thiamine, riboflavin and moisture determinations. Carotene analyses were completed on the day carrots were delivered.

Three varieties of peas were studied, Thomas Laxton, Pride and Shasta. The Thomas Laxton and Shasta peas were harvested at a satisfactory stage of maturity; the Pride peas were slightly more mature. Approximately 500 gram samples of fresh peas or drained canned peas were taken at random from laboratory samples. These were sealed in cellophane bags, quick frozen and held for carotene and moisture analysis. Carotene and moisture determinations were made on subsamples taken at random from the 500 gram laboratory sample.

Carotene analyses were made on one lot of fall planted upland grown spinach and one lot of muck grown

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TABLE 1

Carotene values for carrots (Red Cored Chantenay)

Method of Holding	Time of Holding	Raw					Canned [c]				
		Percent Solids [d]	Carotene Values				Percent Solids [d]	Carotene Values			
			Moist Basis [c]		Dry Basis			Moist Basis		Dry Basis	
			Mg./100 gm.	Percent of sample at harvest	Mg./100 gm.	Percent of sample at harvest		Mg./100 gm.	Percent of sample at harvest	Mg./100 gm.	Percent of sample at harvest
At harvest	....	11.35	11.35	....	100	....	8.55	10.26	90	120	120
Canned on day after taken from storage											
Outdoors in straw	1 week	10.54	12.38	109	118	118	8.08	12.08	106	150	150
	1 month	11.01	12.65	111	115	115	8.32	12.35	109	148	148
	3 months	9.70	9.97	88	103	103	8.52	10.44	92	122	122
Cold storage in crates	1 week	11.39	11.89	105	104	104	8.43	12.40	109	147	147
	1 month	11.08	11.63	102	105	105	8.32	12.56	111	151	151
	3 months	11.17	11.35	100	106	106	8.60	11.59	102	135	135
Canned one day after taken from storage											
Outdoors in straw	1 week	10.42	11.06	97	106	106	8.56	10.75	95	126	126
	1 month	9.71	12.52	110	129	129	8.03	12.79	113	159	159
	3 months	10.87	11.90	105	109	109	8.32	10.42	92	125	125
Cold storage in crates	1 week	10.77	10.54	93	98	98	8.46	11.02	97	130	130
	1 month	11.82	12.91	114	109	109	9.02	13.18	116	146	146
	3 months	11.77	11.15	98	95	95	10.18	12.40	109	122	122

[c] Values average of eight replications. [d] Values average of duplicate determinations. [e] Entire can contents.

spinach. When delivered to the cannery, the muck grown spinach was in very good condition; however, the upland spinach was held for 15 hours in the rain before delivery to the cannery and was of poor quality. Thus precanning holding times for the two varieties of spinach are not comparable. Seven random samples of laboratory lots of spinach were carefully weighed for carotene and moisture analysis. Carotene samples were

TABLE 2

Carotene values for peas

Variety	Method of Holding	Holding Time Hrs.	Raw					Canned [b]				
			Percent Solids [a]	Carotene Values				Percent Solids [a]	Carotene Values			
				Moist Basis		Dry Basis			Moist Basis		Dry Basis	
				Mg./100 gm.[a]	Percent of field sample	Mg./100 gm.	Percent of field sample		Mg./100 gm.[a]	Percent of field sample	Mg./100 gm.	Percent of field sample
Pride	Field sample	....	21.12	0.42	....	1.98	....	....	....	....	....	....
	Prompt handling	1.3	21.09	0.43	103	2.03	102	19.94	0.51	122	2.54	128
	Shade	4.8	21.32	0.45	107	2.09	106	21.81	0.45	108	2.07	104
		10.3	21.80	0.38	91	1.73	87	18.77	0.54	128	2.89	146
	Washed, shade	4.8	19.65	0.30	73	1.55	78	19.51	0.45	107	2.30	116
		10.3	20.40	0.38	91	1.87	94	19.61	0.50	120	2.55	129
	Running water	10.3	18.08	0.40	97	2.24	113	17.92	0.50	119	2.78	140
	Cold storage	10.3	21.59	0.44	105	2.03	102	18.42	0.44	106	2.41	122
		26.0	22.43	0.42	100	1.87	94	17.93	0.44	106	2.46	124
		48.3	21.66	0.38	91	1.76	89	18.85	0.48	116	2.57	130
	Washed, cold storage	10.3	20.08	0.39	95	1.97	100	18.73	0.49	116	2.60	131
		26.0	20.61	0.40	96	1.95	98	18.02	0.49	118	2.73	138
		48.3	20.90	0.34	82	1.64	83	18.76	0.48	116	2.57	130
	Snow ice	26.0	22.03	0.36	85	1.61	81	18.23	0.47	113	2.58	130
		48.3	20.33	0.39	94	1.94	98	17.52	0.44	106	2.53	128
Shasta	Field sample	....	22.74	0.50	....	2.21	....	....	....	....	....	....
	Prompt handling	1.2	21.30	0.45	90	2.12	96	21.32	0.50	99	2.34	106
	Shade	4.4	22.99	0.47	92	2.03	92	16.88	0.51	101	3.02	137
		7.9	21.31	0.46	91	2.16	98	17.88	0.49	97	2.72	123
	Washed, shade	4.4	20.82	0.40	80	1.94	88	19.47	0.52	104	2.69	122
		7.9	20.67	0.40	80	1.94	88	....	....	....	....	....
	Unvined	17.3	21.33	0.42	84	1.99	90	19.33	0.47	94	2.45	111
	Running water	7.9	18.31	0.41	82	2.24	101	16.83	0.50	99	2.96	134
	Cold storage	7.9	22.58	0.44	88	1.95	88	17.86	0.52	103	2.89	131
		24.2	21.60	0.41	81	1.88	85	18.95	0.51	101	2.70	122
	Washed, cold storage	7.9	21.47	0.41	81	1.89	86	18.08	0.56	111	3.08	139
		24.2	21.39	0.44	88	2.06	93	18.47	0.54	106	2.90	131
	Snow ice	24.2	20.38	0.44	88	2.16	98	18.88	0.51	102	2.70	122
Thomas Laxton	Field sample	....	19.92	0.42	....	2.09	....	....	....	....	....	....
	Prompt handling	2.3	20.39	0.36	86	1.79	86	17.32	0.44	106	2.54	122
	Shade	5.5	19.06	0.37	88	1.93	92	....	....	....	....	....
	Washed, shade	5.5	19.48	0.31	74	1.59	76	17.45	0.51	122	2.91	134
	Unvined	4.3	20.33	0.30	71	1.45	69	....	....	....	....	....
	Running water	9.3	17.67	0.34	81	1.90	91	14.11	0.46	112	3.30	158
	Cold storage	9.4	20.18	0.36	86	1.77	85	16.15	0.48	115	2.97	142
		22.8	20.34	0.36	86	1.75	84	17.71	0.44	106	2.33	112
		46.8	19.19	0.24	57	1.23	59	16.84	0.47	113	2.79	134
	Washed, cold storage	9.4	17.52	0.36	88	2.05	98	17.17	0.46	110	2.67	128
		22.8	19.19	0.24	58	1.25	60	14.16	0.43	104	3.05	146
		46.8	19.56	0.34	82	1.75	84	16.62	0.47	113	2.82	135
	Snow ice	22.8	18.61	0.30	73	1.63	78	15.20	0.40	95	2.61	125
		46.8	18.96	0.33	80	1.76	84	16.15	0.43	103	2.65	127

[a] Values average of five replications. [a] Values average of duplicate determinations. [b] Entire can contents.

TABLE 3

Carotene values for raw spinach [1]

Method of Holding	Holding Time Hrs.	Upland Grown			Muck Grown				
		Percent Solids [1]	Carotene Values		Percent Solids [1]	Carotene Values			
			Moist Basis	Dry Basis		Moist Basis		Dry Basis	
			Mg./100 gm.	Mg./100 gm.		Mg./100 gm.	Percent of field sample	Mg./100 gm.	Percent of field sample
Field sample	....	....	....	....	6.49	2.93	....	45.1	....
Prompt handling	2				7.55	4.15	142	54.8	122
Overnight in field	17	7.62	4.83	63.4					
Shade, basket	7				7.22	3.91	133	54.2	120
	21	8.01	5.02	62.7					
	24				6.78	3.62	124	53.4	118
	37	6.92	4.03	58.2					
	72				6.99	3.84	131	54.9	122
Shade, pile	24				6.76	3.75	128	55.5	124
	37	7.74	4.08	52.7					
Cold storage	24				6.77	3.38	115	49.9	111
	37	8.09	3.98	49.2					
	61	7.65	4.24	55.4					
	72				5.94	3.10	106	52.2	116
	133	7.18	3.39	47.2					

[1] No canned spinach was analyzed. [1] Values average of duplicate determinations.

sealed in small cellophane bags, quick frozen and held until analyses were completed. The total spinach content of these bags was used for carotene determinations. Frozen vegetable samples were held in deep freeze at —15° C.

The carotene content of the vegetables is given in Tables 1-3. Data are shown for raw, canned and stored vegetables on both moist and dry weight basis.

Carotene values reported are averages of eight replicates of raw carrots, five of raw peas and duplicate determinations on raw spinach, canned carrots and canned peas. Percent solids and differences from the fresh are indicated. Holding times indicated for various storage or holding methods refer to the time between harvest and canning. Carotene was determined by the Petering, Wolman and Hibbard *(8)* procedure using a Waring blendor for extracting vegetable carotene into absolute ethyl alcohol. Carotene values were read from a standard curve made with a mixture of 90 percent beta and 10 percent alpha carotene.

Results and Discussion

Carrots. There is little variation in the carotene content of the freshly harvested, stored and processed carrots. Carrots stored outdoors in straw and in crates in cold storage up to one month gave slightly higher (2-11 percent) carotene values than freshly harvested carrots. At the end of three months' storage the outdoor stored carrots were 12 percent lower than the original sample and cold stored carrots gave the same carotene value as the freshly harvested vegetable.

When compared on the moist weight basis, all canned carrots processed after previous outdoor or cold storage gave higher carotene values (2-29 percent) than canned carrots processed at harvest. Carrots canned after three months' storage tended to contain slightly less carotene than those stored one week or one month. Compared on the drained moist weight basis, canned carrots were as high or higher in carotene than the fresh or stored carrots. Carrots taken from storage and held one day before canning gave slightly different carotene values than carrots analyzed or canned the day taken from storage; however, no definite pattern was evident.

Peas. The field sample of Shasta peas contained 19 percent more carotene than the field sample of the other two varieties. Lots of Shasta peas analyzed prior to canning ranged from 0.40 to 0.47 mg. carotene per hundred grams; similar samples of the Thomas Laxton variety ranged from 0.24 to 0.37 mg. and Prides ranged from 0.30 to 0.45 mg.

In general retention of carotene in stored samples of Pride peas was greater than in the other two varieties. Minimum retention (73 percent) occurred when this variety was washed and held five hours in the shade. There was 82 percent retention of carotene in peas washed and held in cold storage 48 hours and 85 percent in peas held 26 hours in snow ice. Other methods of holding peas of this variety caused less than 10 percent change in the carotene content of the vegetable.

Carotene values for Shasta peas held prior to canning were 8 to 20 percent lower than the field sample value. The poorest retentions (80 percent) of carotene were in the samples held in the shade for four and eight hours after washing. There were retentions of 81 to 82 percent when peas were held in cold storage one day, in running water eight hours or washed and held in cold storage eight hours.

When compared with the other two varieties carotene retention was consistently less in the Thomas Laxton peas. Here a minimum retention of 57 percent occurred when the peas were held in cold storage 47 hours. Only 58 percent of the carotene remained after 23 hour storage of washed cold stored peas. Other retention values ranged between 88 and 73 percent.

Canned peas compared favorably in carotene concentration with the field sample or stored product on the basis of drained moist weight. Most canned samples were higher in carotene than peas from the same fresh or stored lots. Ranges of carotene values of canned peas were Pride 0.44 to 0.54 mg. per 100 grams, Thomas Laxton 0.40 to 0.51 mg. per 100 grams and Shasta 0.47 to 0.56 mg. per 100 grams.

Spinach. Upland grown spinach showed a slight increase in carotene content when held in baskets in the shade for 21 hours; however, after 37 hours storage either in the shade in piles or baskets, the spinach was lower in carotene than the first sample analyzed. When this spinach was held in cold storage for 37, 61 and 133 hours, there was apparently some loss of carotene. Compared on the moist weight basis the upland spinach held in cold storage for 133 hours was 30 percent lower in carotene than the sample assayed when the spinach was delivered to the cannery.

Carotene values for muck grown spinach were lower than samples of the upland grown crop. All cannery samples delivered to the laboratory gave higher carotene values (6 to 42 percent) than the field sample. Muck grown samples held in cold storage 24 to 72 hours contained less carotene per unit weight than samples held by other methods.

Several carotene values reported here for stored or processed carrots, peas and spinach are higher than carotene values for the comparable fresh vegetable sample. Similar findings have been reported by other laboratories and are summarized by Porter, Wharton and Bennett *(9)*. These authors weighed portions of fresh spinach into small sealed pliofilm bags which were impervious to water and determined the amount of carotene of the entire content of the bag. The carotene of the cooked sample did not differ from that of the uncooked sample when the computation was based on the precooked weight of the sample or the dry weight of the raw sample. Determination of dry weight on comparable cooked samples and the computation of the carotene content of the drained cooked spinach in relation to the total solids of the cooked product yielded carotene values more than 30 percent greater than the fresh values. An insignificant portion of the difference could be accounted for by including the solids of the cooking liquid in the calculation. No carotene was found in the cooking liquid. The authors postulate that dry weight is a variable measure dependent upon the physical

structure of the product dried and point out that small variations in dry weight may account for wide variations in the amount of a nutrient occurring in as dilute concentrations as carotene.

The greatest apparent increases in the carotene of the vegetables analyzed in this study were in the canned products. It is suggested that the changes in colloidal structure resulting from cooking caused the carotene values of the canned product to appear high in relation to the fresh vegetable because of differences in the total solids measured under the standard conditions of determining moisture content of food samples. Total solids were determined on both the drained vegetable and the liquids of the canned sample.

The effect of prolonged storage of the raw sample on the determination of total solids has not been studied. However, storage samples varied widely in texture and it is not unreasonable to assume changes in the plant colloids as a result of pre-processing storage.

Summary

The effects of accepted commercial canning practices on the carotene content of carrots, peas and spinach are reported. Data presented indicate that various holding methods imposed prior to canning affect the apparent carotene level of vegetables studied. The carotene content of the canned vegetables compares favorably with that of the freshly harvested vegetable.

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Industrial and Legal Viewpoints

Independent Scientific Laboratories Serve the Food Industry[a]

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Several types of services rendered the Food Industry by independent consulting laboratories are discussed. Circumstances are described and cases are cited in which these laboratories served Food Industry effectively and economically.

Independent scientific laboratories in this country are an integral part of our national economy and are vigorous exponents of the American system and of the principles of private enterprise. As commercial organizations, they are dedicated to assist the development of American industry and make specific contributions to the communities in which they are located.

At present [b] there are approximately 300 such laboratories staffed with more than 5000 scientists, the majority of whom are college and university trained chemists, bacteriologists, food technologists, engineers, physicists and other scientists. These workers are experienced in meeting the problems of industry and are familiar with a wide variety of products of manufacture, from raw materials to finished products. This group of independent commercial laboratories should not be confused with the so-called "captive" industrial laboratories owned and operated by individual companies.

Approximately 80,000 clients use these services annually and in this way the laboratories render a service which directly relates to the continued employment of 20,000,000 workers employed by these 80,000 clients. It is of interest to note that a majority of these 300 laboratories serve members of the food industry.

Independent research laboratories are the logical place for small and medium sized businesses to come because these organizations have needed equipment,

[a] Presented before the Ninth Annual I. F. T. Convention, San Francisco, California, July 11, 1949.

[b] Independent Scientific Laboratories in our National Economy, A Report to the President's Scientific Research Board, April 15, 1947, the Washington Liaison Service, Washington, D. C.

personnel and scientific "know-how." If the research problem is temporary, sponsors will find these organizations to be the most economical place to conduct their research. When the research problem is completed, expense ceases. Sponsors have to make no investment in personnel or equipment, and can terminate the work whenever they want to. They may start new problems on short notice, and may have their work done by people who are trained in doing research and making investigations with the objective of a practical application of the findings.

Practical viewpoint and experience are most important. Some processes and products have been developed in the pursuit of pure scientific research, which at first appeared to be the answer to a sponsor's prayer. However, they could not be utilized or marketed because they were uneconomical. A process may be theoretically sound and demonstrable in the laboratory, but in pilot plant or commercial scale production, certain "bugs" may show up, such as low yields or uneconomical operating costs. New products may look promising but the ingredients may be too costly to produce a finished item with a satisfactory margin of profit for the manufacturer, wholesaler and retailer. The development of a superior product is of little commercial value to the client, if it cannot be produced and marketed at a price the consumer is able and willing to pay.

Independent scientific laboratories have served and are serving the food industry in many ways. Some of the more common ones are classified below:

1. Research and development of new products and processes and improvement of existing products;
2. Quality control of production;
3. Special "trouble-shooting" problems;
4. Product liability cases and technical testimony;
5. Establishment of label claims, representation before regulatory agencies and preparation of proposals for seals of acceptance;
6. Scoring and standardization of finished products;
7. Plant inspections of sanitary conditions;
8. Study of the toxicological aspects of foods;
9. Problems relating to agriculture as pertaining to food production; and
10. Utilization of by-products and disposal of waste products.

Research is often directed toward the development of new food products, better quality of present ones, and improvement of existing methods of food production. This particular field of activity holds great importance. It is one which appears to be most essential for the food industry during times of economic recession and increased competition. Enlarging consumer acceptance of a line of prepared foods through improved quality with little or no increase in the cost of production may be the difference between financial success and failure. In this day of aggressive research in all fields of human endeavor, no food producer can afford to be without research services.

Although a leading industrialist of this country, Mr. Paul G. Hoffman, was not speaking[c] specifically of foods, the food industry might well reflect upon his statement: "Small businesses should engage the services of properly qualified laboratories to assist them in research. Today 50% of all commodities which are manufactured and sold did not exist in their present form 25 years ago and I predict that in 10 years, 25% of all products manufactured and sold, will be those not even existing today."

Organoleptic panel scoring is one of the effective research tools used by laboratories today. The value of this depends upon the degree of precision and reproducibility attained. Quantitative evaluation of appearance, aroma, color, flavor and texture of food products has been correlated to a high degree with actual consumer acceptance as determined by suitable consumer surveys. Although advertising, method of distribution, packaging and appearance may be of paramount importance in the initial sales of a food, repeat sales depend upon consumer preference.

One food producer who was marketing a highly competitive canned food found that initial sales were satisfactory but repeat sales were poor. An organoleptic test revealed that one spice ingredient in the product imparted a flavor which made the product unacceptable to a majority of the taste panel. The producer stated that his production superintendent, who had developed the product, insisted that he liked the particular flavor and there was no good reason why the consumers wouldn't like it too. But they didn't. The offending ingredient was removed from the formula and in due time sales increased.

Accelerated storage tests are easy to conduct, but are overlooked by many producers of new food products. They are so anxious to get into the market, they overlook the possibility of what might happen to their new "baby" under adverse shelf-life conditions. A producer has his plant in a part of the country where relatively low temperatures prevailed during most of the year. He hadn't stopped to realize that his product was going into areas where relatively high year around temperatures prevailed. The flavor changes which developed in his merchandise in these areas made it unacceptable. A heavy financial loss resulted through replacements after he had properly stabilized his product.

Quality control generally involves both raw ingredients and finished food product. Severe economic losses have occurred when this control was neglected. Cases of such are legion. Large companies maintain their own laboratories. However, the smaller food producers have available the services of the independent laboratory which can conduct required laboratory work or can supervise (or staff) a small laboratory at the producer's plant.

Another phase of this work involves responsibility vested in the independent laboratory by a client, such as a buyer or commodity loan department of a bank, who wishes analyses and examinations made upon lots of food which are under consideration for collateral or purchase. The matter of acceptance or rejection is important to all concerned.

One case involved a product which was sold on the basis of protein content. The producer generally was successful in "unloading" his low protein, substandard

[c] Private Enterprise in the Post War World, an address delivered March 8, 1949 before The Rotary Club of Los Angeles.

lots upon small buyers who did not have laboratory analyses run upon purchases. Perhaps this is not a typical case, but it does illustrate that buyers should seek competent laboratory services.

Numerous and varied are the problems encountered by food producers. Production can be proceeding normally when without warning, a serious problem may arise. Prompt assistance is required and the independent laboratory generally can give it.

Insurance adjusters consult independent laboratories for reliable and impartial service in determining facts. It is fortunate that only a small minority of consumers are dishonest. Some cases which have come to the writer's attention are amusing. However, claims by consumers of contaminated foods and beverages have often been substantiated by laboratory examinations. Portions of rodents, insects, lubricating grease, nuts, bolts and many other foreign materials have been identified.

The sequel to the above may be court appearances to provide technical testimony. Such legal services often are sought in other matters involving the food industry, unrelated to consumer claims for damage.

Within recent years greater emphasis has been placed upon the preventive service of laboratories in carrying on periodic plant inspections of sanitary conditions. It would seem advisable that staff members assigned for this work should have a background of industrial bacteriology and food technology. The recommendations of such inspectors may involve such matters as adequate rodent and pest control, proper water treatment, suitable refrigeration, plant clean-up procedures, the proper use of germicides and cleaners and good over-all production procedures. Carrying out such recommendations has been of great value to many of the food industry. The proverbial "ounce of prevention" is just as true here as it is in other matters.

Recent emphasis has been placed upon the study of the toxicological aspects of foods by one of the independent laboratories. Animal experimentation has been employed in studying the safety of the use of chlorine dioxide in the bleaching and maturation of flour to supplant the nitrogen trichloride treatment process which has been in use in the past. While the nitrogen trichloride treatment has never been shown, at least to date, to produce toxic results in man despite the most elaborate attempts to induce it, its adverse effects on other species, especially the dog, has resulted in its prohibition. The chlorine dioxide treatment has been found to be completely innocuous.

The use of chemical agents in foods as preservatives, antioxidants, emulsifying agents, etc., has expanded considerably in recent years. One independent laboratory has been studying the toxicological aspect of polyoxyethylene derivatives when used as softening agents in bread. The results of this work were reported at hearings of the Federal Food and Drug Administration designed to determine the acceptability of such softening agents in the definition and standard for bread.

Another independent laboratory investigation involved a study of bactericidal and fungicidal agents incorporated in paper pulp and coating to inhibit bacterial growth. The possibility of imparting injurious properties to foods wrapped in such materials is of great importance.

Recent instances of poisoning alleged to have resulted from the use of salt substitutes by cardiac patients on "salt-free" diets has led to the investigation by an independent laboratory of this subject from the point of view of the relation between the sodium and/or potassium level of the diet and the toxicity of supplemental lithium or potassium salts.

One of the most interesting research projects being carried on by an independent laboratory group involves a consideration of man's oldest industry, agriculture, and his newest discovery, the development of atomic energy. More than half of the approximately 2 billion people in the world today do not get enough to eat, and the population is growing at the rate of about 20 million each year. Food production is not increasing as fast as population. A prime need of mankind is a greater output of agricultural products.

Although research with isotopes has not as yet brought larger food yields, perhaps it will in time. Radioisotopes of a score of elements useful in plant and animal research, such as carbon, phosphorus, sulfur, calcium and potassium, today are plentiful and inexpensive.

The fundamental question of agriculture is how plants grow; how green leaves in sunlight photosynthesize carbohydrates from water and carbon dioxide; how roots pick up minerals and organic matter from the soil; how these many substances are distributed in and used by the plant in its growth. The majority of such questions have gone unanswered because scientists have had no means of observing the intricate processes and the minute quantities of materials. But, the tracer technique has changed this. For example, using radioisotopes, observers have been able to follow through the soil, into the rootlets, and to their final disposition in the plant, minerals such as zinc, copper, and manganese, all of which are available to the plants in amounts of less than an ounce per acre.

Through this new scientific tool, much information is being gained regarding fertilizers and thus directly the production of agricultural products. Another phase of this research involves the general recognition that certain elements in small amounts are essential for animal health, having a regulatory function perhaps similar to that of the vitamins. The bodily requirements for these trace elements are so minute that their function cannot be studied with ordinary chemical methods. The use of radioisotopes offers unique possibilities for the investigation of the role and function of these minerals in animal metabolism. It is anticipated that these studies will contribute to improved animal production, as well as improved nutrition for man.

It is hoped that this brief presentation has indicated some of the services rendered the food industry by independent laboratories throughout this land and how these services might be increased. These laboratories believe that the spirit of independence in America is necessary to the maintenance of our democratic way of life and they are so dedicated.